Assessment of treatment failure with Artemisinin combination therapy (ACT) in management of falciparum malaria in a tertiary care centre in South India

A dissertation submitted in partial fulfilment of the rules and regulations for MD General Medicine examination of the Tamil Nadu Dr.M.G.R Medical University, Chennai, to be held in April 2015

DECLARATION

This is to declare that this dissertation titled "Assessment of treatment failure with Artemisinin combination therapy in management of falciparum malaria is my original work done in partial fulfilment of rules and regulations for MD General Medicine examination of the Tamil Nadu Dr.M.G.R Medical University, Chennai to be held in April 2015.

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CERTIFICATE

This is to certify that the dissertation entitled, "Assessment of treatment failure to Artemisinin combination therapy (ACT) in a tertiary care centre in South India" is a bonafide work done by

Dr.T.Angel Miraclin Jebakumari

towards the partial fulfilment of rules and regulations for MD General Medicine degree examination of the Tamil Nadu Dr.M.G.R Medical University, to be conducted in April 2015.

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ACKNOWLEDGEMENT

This dissertation would be incomplete without expressing my gratitude to the people involved in its conceptualisation and completion.

My sincere gratitude to my guide, *Dr.Priscilla Rupali*, Professor of Medicine and Infectious diseases, for the mentorship and guidance throughout this process, since its conception to completion.

I thank *Dr.Joy Mammen*, Professor of Clinical Haematology and Transfusion Medicine, for his guidance in conducting the parasite clearance studies.

I thank *Dr.Binu Mathew*, Professor of Clinical Pharmacology, for the constant support and words of encouragement throughout this new venture in pharmacokinetic studies, and her team for the development of the drug assay and assistance in conducting the studies.

I thank *Mrs. Lilly Selvam* and *Mrs. Daisy* and the Clinical Pharmacology team, for their assistance in the pharmacokinetic studies.

I thank *Mrs.Visalakshi*, Department of Biostatistics for her expertise in the statistical analysis.

I also express my sincere gratitude to my teachers particularly *Dr.Anand Zachariah*, Professor and Head of Medicine for his valuable suggestions and *Dr.Thambu David*, Professor of Medicine for effectively inculcating the principles and ethics of research into our curriculum, and my colleagues in the Department of Medicine who helped in patient recruitment.

I thank my parents *Mr.Thirugnana Kumar* and *Mrs. Regina*, Sister *Ms.Annie Elizabeth*, for their presence, constant support and words of encouragement.

I thank God for this opportunity and by whose grace this was possible

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ABSTRACT

TITLE OF THE ABSTRACT: Assessment of treatment failure with Artemisinin combination therapy in management of falciparum malaria.

DEPARTMENT: General Medicine

NAME OF THE CANDIDATE: T.Angel Miraclin Jebakumari

DEGREE AND SUBJECT: MD General Medicine

NAME OF THE GUIDE: Dr.Priscilla Rupali

KEY WORDS: Treatment failure, Artemisinin combination therapy (ACT), falciparum malaria

WORD COUNT: 471 words

OBJECTIVES:

The study was done to assess the incidence of treatment failure to Artemisinin combination therapy (ACT) by studying the clinical response and the parasitological clearance in patients with falciparum malaria. The drug levels were assessed and the basic drug pharmacokinetic variables were correlated with treatment response.

METHODS:

In a prospective cohort study from October 2012 to June 2014, fifty four slide confirmed cases of falciparum malaria on ACT were enrolled. Clinical response was characterised by fever clearance time (FCT), assessed by measurement of body temperature till defervescence. Parasitological response was measured by parasite clearance time (PCT), which is the time to the first negative peripheral smear, with undetectable asexual forms. Seventeen patients with severe malaria requiring parenteral therapy were treated with 2.4 mg/kg intravenous artesunate according to WHO guidelines 2010 for treatment of severe malaria. Venous blood samples were obtained on day 3 of ACT at multiple time points (0 - 240 minutes) after artesunate dose and the plasma concentrations of artesunate and dihydroartemisinin were measured using liquid chromatography-tandem mass spectrometry. The observed maximum plasma

concentrations, four hour exposure and time to maximum concentrations were reported. The host factors, severity of infection, organ dysfunction and drug concentrations were correlated with treatment response.

Parasite clearance times and fever clearance times were assessed by means of survival analysis with use of the Kaplan Meier method, and data analysis was done by means of student's t test, the chi – square test or Mann- Whitney U test, as appropriate. Boot strap analysis was done to correlate factors affecting treatment response in a larger population.

RESULTS

The proportion of patients with early treatment failure as defined by persistent parasitemia on day 3 of ACT was 25.5%. Recrudescence as defined by relapse of fever post treatment with ACT was 9.3%.The median parasite clearance time was estimated to be 36 hours (95%CI: 27.08 – 44.91). The median fever clearance time was estimated to be 24 hours (95% CI: 18.69 – 29.30). Among the patients with delayed parasite clearance, the mean clearance time was found to be 77.53 hours and in those with delayed fever defervescence, the mean fever clearance time was 84.6 hours. Higher age, acute kidney injury, acute hepatic injury, metabolic acidosis and hyperparasitemia were found to be risk factors in univariate analysis. Basic pharmacokinetic variables assessed were similar between responders and non responders. Multivariate logistic regression analysis showed higher age to be a risk factor. Boot strap analysis did not reveal any association between host factors or drug concentrations to the treatment response.

CONCLUSIONS

The susceptibility of Plasmodium falciparum to artemisinin combination therapy is declining in South India resulting in early treatment failure. The pharmacokinetics of artesunate and dihydroartemisinin was not different between the responders and non responders suggesting that a parasite factor is contributing to decreased susceptibility to ACT. Molecular studies are required to confirm resistance.

1. INTRODUCTION

Anti malarial drug resistance is defined as persistence or recurrence of malarial parasites after appropriate drug treatment(1). Artemisinin based combination therapy (ACT) is the current recommended first line anti malarial therapy in management of severe malaria(2).The changes in parasite susceptibility to ACT detected by therapeutic efficacy studies is essential for tracking artemisinin resistance(3).Therapeutic efficacy studies aid in the identification of proportion of patients who are parasitemic on day 3,which is the most strong and reliable factor to identify treatment failure(1,3). Rates of treatment failure exceeding 10% warrant a change in the antimalarial treatment policy in the region(4).

Infection with *Plasmodium falciparum* species sensitive to ACT clear within two days of initiation of therapy as compared to resistant parasites where a delay in clearing of the parasites was observed .The decreasing response to artemisinin was first observed in Cambodia – Thailand border which has since become the epicentre of emergence and spread of artemisinin resistance(5,6). India shares a common border with Thailand and Bangladesh which have thick forests and landscape favouring vector transmission compounded with poor accessibility to health care, which could potentiate entry of resistance from the Greater Mekong Region(7).Delayed susceptibility to artesunate and sulphadoxine based combination therapy has been recently reported in three eastern states of Arunachal Pradesh, Mizoram and Tripura(8).We have been observing a trend of delayed defervsecence of fever and persistence of parasites in our population. Hence, this study was designed to identify the incidence of treatment failure with Artemisinin combination therapy (ACT) among patients with falciparum malaria presenting to Christian Medical College, Vellore, a tertiary care centre in South India.

2. AIM

To assess treatment failure with Artemisinin combination therapy (ACT) in management of *Plasmodium falciparum* malaria in patients presenting to Christian Medical College, a tertiary care hospital in South India.

3. OBJECTIVES

1) To determine the incidence of treatment failure in *falciparum* malaria in patient treated with ACT.

2) To assess the clinical response (CR) and parasitological clearance (PC) of *Plasmodium falciparum* in patients treated with Artemisinin combination therapy (ACT).

3) To determine the serum concentrations of artesunate and dihydroartemisinin and correlate clinical response with the same.

4) To study the variability in pharmacokinetic parameters of artesunate and dihydroartemisinin between patients who respond to therapy and in patients with early treatment failure.

4. REVIEW OF LITERATURE

4.1 MALARIA - EPIDEMIOLOGY

Malaria is a tropical disease which is caused by five species of genus *Plasmodium* causing human infection. The species are mainly *Plasmodium falciparum, ovale, vivax, malariae and knowlesi*.Infections caused by *falciparum* and *vivax* are of great public health concern in the tropical countries. Active transmission is documented in 104 countries and are presently endemic for malaria as of WHO global malaria report 2013(2).Africa contributes to 80% of the malarial deaths ,of which 77% are in the paediatric age group. Malaria is transmitted by female Anopheles mosquito. Of the 400 species identified, 30 are vectors of significance in transmission. Two of the widely used antimalarials are derived from plant sources – artemisinin derivative (*Artemisia annua*), Quinine (*Cinchona*) are currently in use in areas endemic for malaria transmission.

4.1.1 TRENDS IN SOUTH EAST ASIA – SUMMARY OF WORLD MALARIA REPORT 2013

Diagnosed malarial infections, confirmed by light microscopy reported in the South east region, during the time span of the year 2000 - 2012 decreased from 2.9 to 2 million.
96% of the reported cases of malaria in 2012 were contributed by the three countries: India (52%), Myanmar (24%) and Indonesia (22%).Considering the Indian scenario, the number of confirmed cases being reported have declined from 2 million in the year 2000 to 1.1 million in the year 2011.The provided national statistics suggest that the country is enroute to achieving a decrement in the incidence of malaria by 50% - 75% by 2015(2). The mortality due to malaria in the region has also decreased from 5500 to 1200 between the time span of 12 years, from 2000 and 2012. India accounts to 42% of malarial deaths in the year 2012.

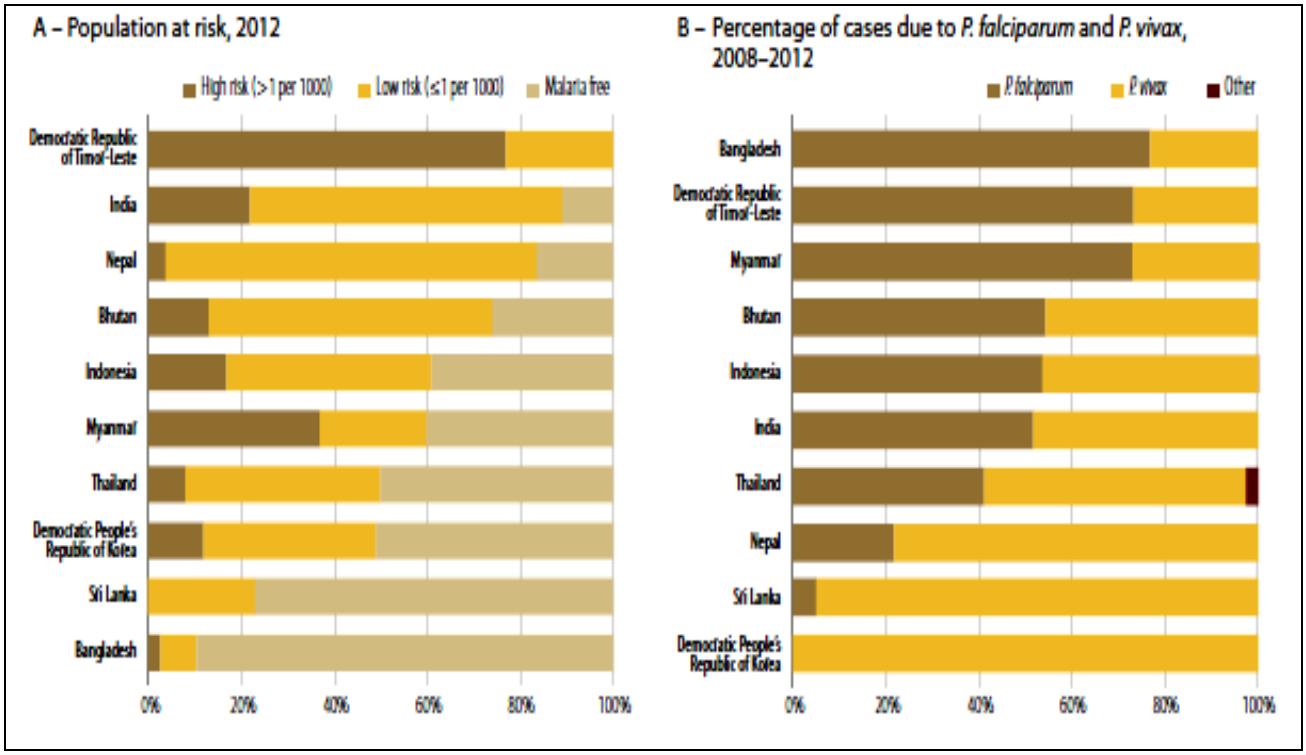


Figure 1 The country wise statistics of malaria [Adapted from WHO malaria report 2013]

4.1.2 THE INDIAN SCENARIO

95% population live in malaria endemic areas and 80% of malaria reported in the country is confined to areas which are hilly terrains, inaccessible to medical care(9). The number of cases has been on a decline from the year 2002(10).50 % of reported cases are caused by *falciparum* species and 50% by *vivax* species. North and north eastern India are endemic for *falciparum* infection. The common anopheles species found include *An. fluviatilis, stephensi, dirus, minimus, annularis and culicifacies.*Artesunate and sulphadoxine- pyrimethamine combination is currently recommended first line treatment. Combination therapy based on quinine is recommended for management of treatment failure.

The case distribution and the surveillance measures are summarised in Figure 2.

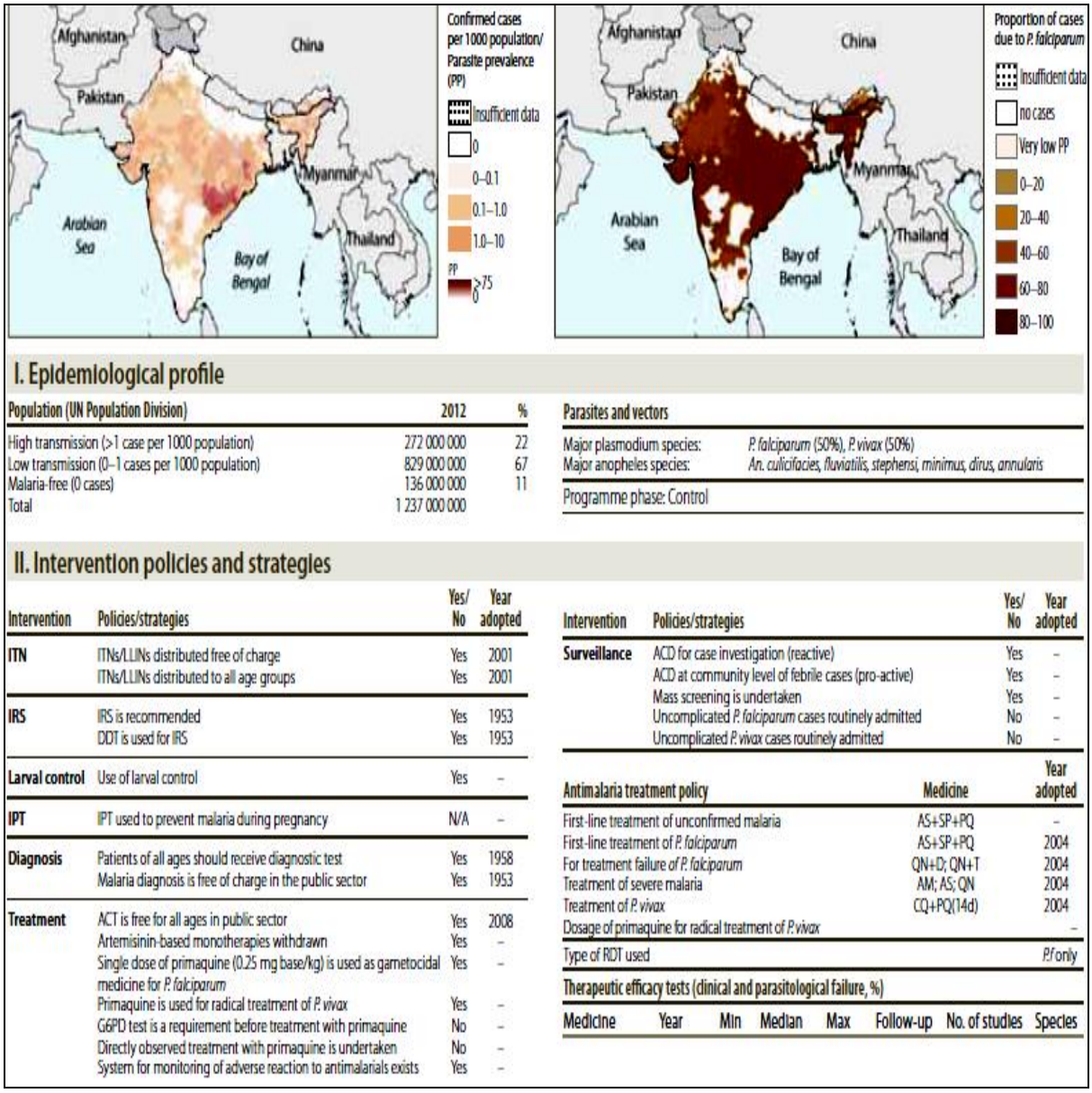
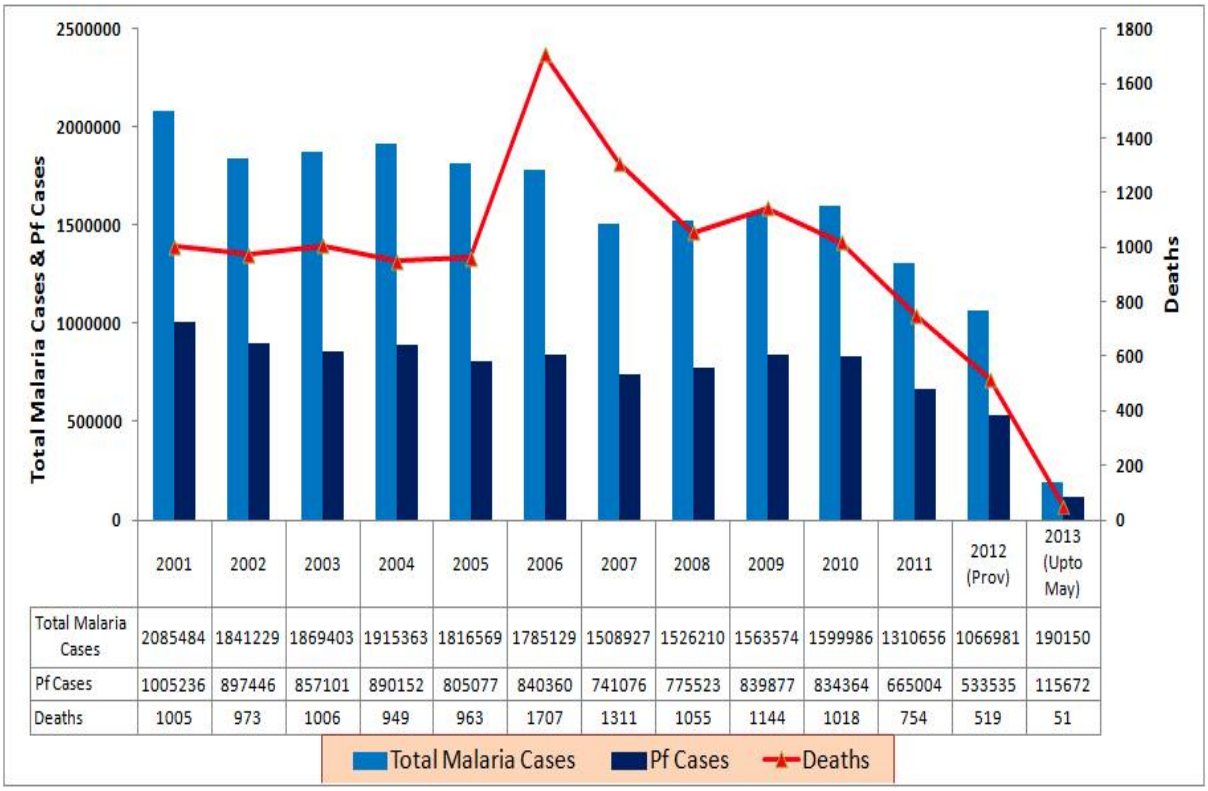


Figure 2 Epidemiology and interventional strategies [adapted from WHO malaria report 2013]

Public sector based interventions by the NMEP have contributed significantly to the decline in incidence of malaria cases. Presently malaria diagnostic tests as well as ACT has been administered free of cost in the public sector. Extensive preventive measures are in place which includes larval control as well as the larval control measures.

The impact of these diagnostic and treatment interventions are summarised in figure 3.



	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012 (Prov)	2013 (Upto May)
Total Malaria Cases	2085484	1841229	1869403	1915363	1816569	1785129	1508927	1526210	1563574	1599986	1310656	1066981	190150
Pf Cases	1005236	897446	857101	890152	805077	840360	741076	775523	839877	834364	665004	533535	115672
Deaths	1005	973	1006	949	963	1707	1311	1055	1144	1018	754	519	51

Figure 3 Impact of interventions and surveillance[Adapted from nvbdcp.gov.in](9)

The national epidemiological indicators also show a marked decline over the last 5 years. Annual Parasite Incidence (API) rate has decreased from 2.12/1000 in 2001 to 0.88/1000 in 2012(10).Slide Falciparum rate has also reduced significantly.

In the state of Tamilnadu, 74% are reported from Chennai the remaining 8.4% are from Vellore, Tuticorin, Erode, Dindigul, Tiruchengode, Salem, Tiruvallur and Tiruvottriyur(11).

In contrary to the national and WHO data, which is reassuring ,a study published in The Lancet in 2010 by Dhingra et al(12) have showed that the malarial deaths are grossly underestimated and they had reported 1,20,000 deaths/year in India, as compared to national statistics which reported the number to be 1230 deaths.

The mortality occurred in geographical areas which are highly endemic for *falciparum* infections.

4.2 THE PARASITE

Transmission in humans occur by the bite of female Anopheles mosquito which harbours the Plasmodium sporozoites .The exoerythrocytic stage consists of the travel of the sporozoites to the invasion of hepatocytes.

In the hepatocytes they multiply exceedingly creating tissue schizonts loaded with merozoites. *P.falciparum* schizonts rupture after a duration varying between 6 – 16 days releasing them into the blood stream.

Erythrocytic cycle begins following the rupture of schizonts, where the released merozoites invade the red blood cells and undergo maturation through ring stages to mature trophozoites to form schizonts (the period of maturation differs between different species).The daughter merozoites continue infecting the available red blood cells, however few of them differentiate to sexual forms or gametocytes (male and female).The sexual forms help in continuation of transmission.

The lifecycle of the parasite is summarised in Figure 4.

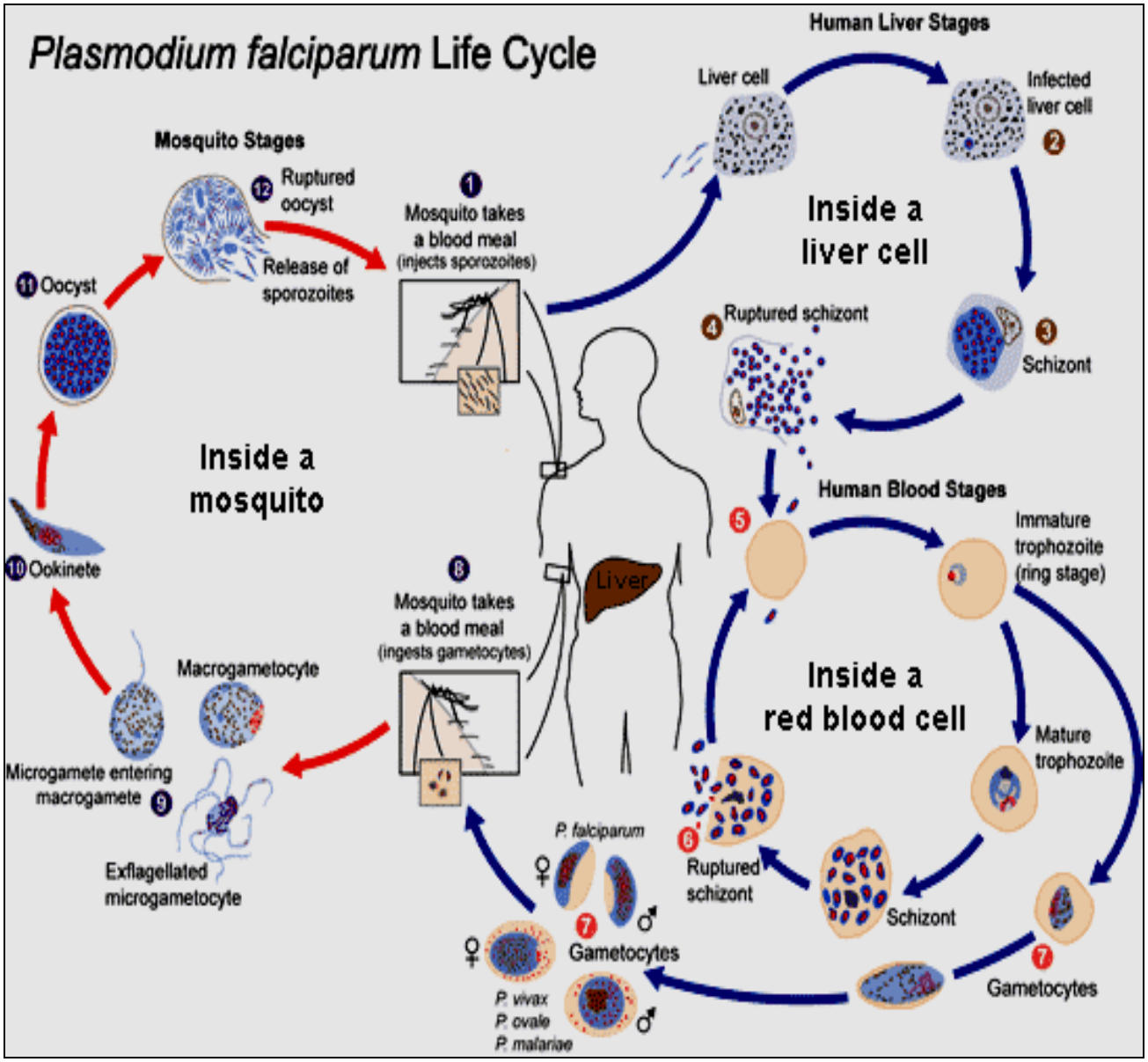


Figure 4: Lifecycle of Plasmodium falciparum [adapted from CDC]

The genome of P.falciparum shows a marked genetic diversity between different geographic regions. Variation of the copy number of genes in the genetic pool predisposes to development of antimalarial drug resistance(13).

4.2.1 PATHOPHYSIOLOGY

Malaria is a disease which involves complex interaction between the host and the parasite, and the clinical presentation of the disease is sequelae of the host parasite interaction. The host parasite interaction and their physiology can be discussed under the following topics:

4.2.1.1 Micro vascular sequestration:

The parasitic forms in the erythrocytes cause formation of sticky knobs which are basically comprised of proteins induced by the parasite itself namely RESA,pfEMP1and pfEMP2.Human proteins like spectrin and actin also contribute to the same(14).Proteins interact with the host endothelial receptors such as CD36,chondroitin sulphate A(CSA),intercellular adhesion molecule-1 (ICAM-1),E-selectin, platelet endothelial cell adhesion molecule-1,vascular cell adhesion molecule-1 (VCAM-1),thrombomodulin (TM), (PECAM-1)and P-selectin(15).The end result of this interaction is intravascular sequestration, which is responsible for most of the manifestations of severe malaria including cerebral malaria as well as severe intravascular haemolysis.

4.2.1.2 Parasite – Host interaction:

The complex interaction of the malarial parasite with host immune system has not been identified. Available evidence suggests that the complex interaction up regulates the cytokines causing a cytokine storm mimicking a sepsis syndrome.

4.2.1.3 Coagulation:

Thrombocytopenia is the most common haematological abnormality in malaria. It occurs as a protective mechanism to compensate for intravascular sequestration of the parasitized red blood cells. Activation of tissue factor occurs during the course of infection , which causes

activation of the coagulation cascade, leading to a consumptive coagulopathy associated with elevated fibrin degradation products (d – dimer)(16).The platelets aggregate with red blood cells leading to thrombocytopenia and the degree of thrombocytopenia can provide the information as to the severity of the disease(16).

4.3 THE 'TISSUE FACTOR MODEL' OF PATHOGENESIS

Currently, the 'Tissue factor Model' is the proposed model of pathogenesis to explain the haematological manifestations(17).It combined the previous two hypothetic models describing pathogenesis namely the 'sequestration hypothesis', which makes the parasitized RBC the prime factor causing the disease manifestations, and the 'cytokine hypothesis', which states that the inflammatory cascade triggered by the parasite causes the damage.

According to the present tissue factor model, the parasitized red blood cells cause endothelial injury, which further leads to activation of tissue factor(17). This further activated the coagulation cascade and incites a state of systemic inflammatory response. The parasitized red blood cells form complex with platelets and the products of coagulation cascade and act as a 'switch' to trigger further immune response, leading to endothelial injury.

The capillary endothelium is affected in all the internal organs and depending on the severity of parasitemia is directly proportional to the severity of multi organ dysfunction. Cerebral malaria and black water fever are a few selected manifestations to prove this current hypothesis.

4.4 DIAGNOSIS OF MALARIA

Parasite based diagnostic methods are the standard of care and reliable. Currently available methods include microscopy and rapid diagnostic tests.

4.4.1 LIGHT MICROSCOPY:

Microscopy helps in diagnosing the infection as well as to identify the species causing the infection. It also helps to quantify the severity of infection using the derived parameter, parasite index. Blood smears are used in diagnosis of malarial infection. The thick smear helps in diagnosis and the thin smears help in identification of the species. Ring-form trophozoites (rings) of *Plasmodium falciparum* are thin and delicate and measures up to a fifth of the diameter of the red blood cell. They contain chromatin dots, found on the periphery of the RBC (appliqué, accolé) (Figure 5a). Gametocytes of *Plasmodium falciparum* are sausage or crescent shaped, and measure about one and half times the diameter of an RBC. The macro gametocytes (female) are darker and stain deep blue when compared to the cytoplasm of the micro gametocytes (male) (Figure 5b). Chromatin is coarser and dark red with pigment in the macro gametocytes. The remnants of the RBC with parasites are called as Laveran's bib (Figure 5c). Schizonts are visualised in hyperparsitemic individuals (Figure 5d).

Parasite density is calculated by a standardised method by counting the number of parasitized erythrocytes per 200/500 white blood cells per field.

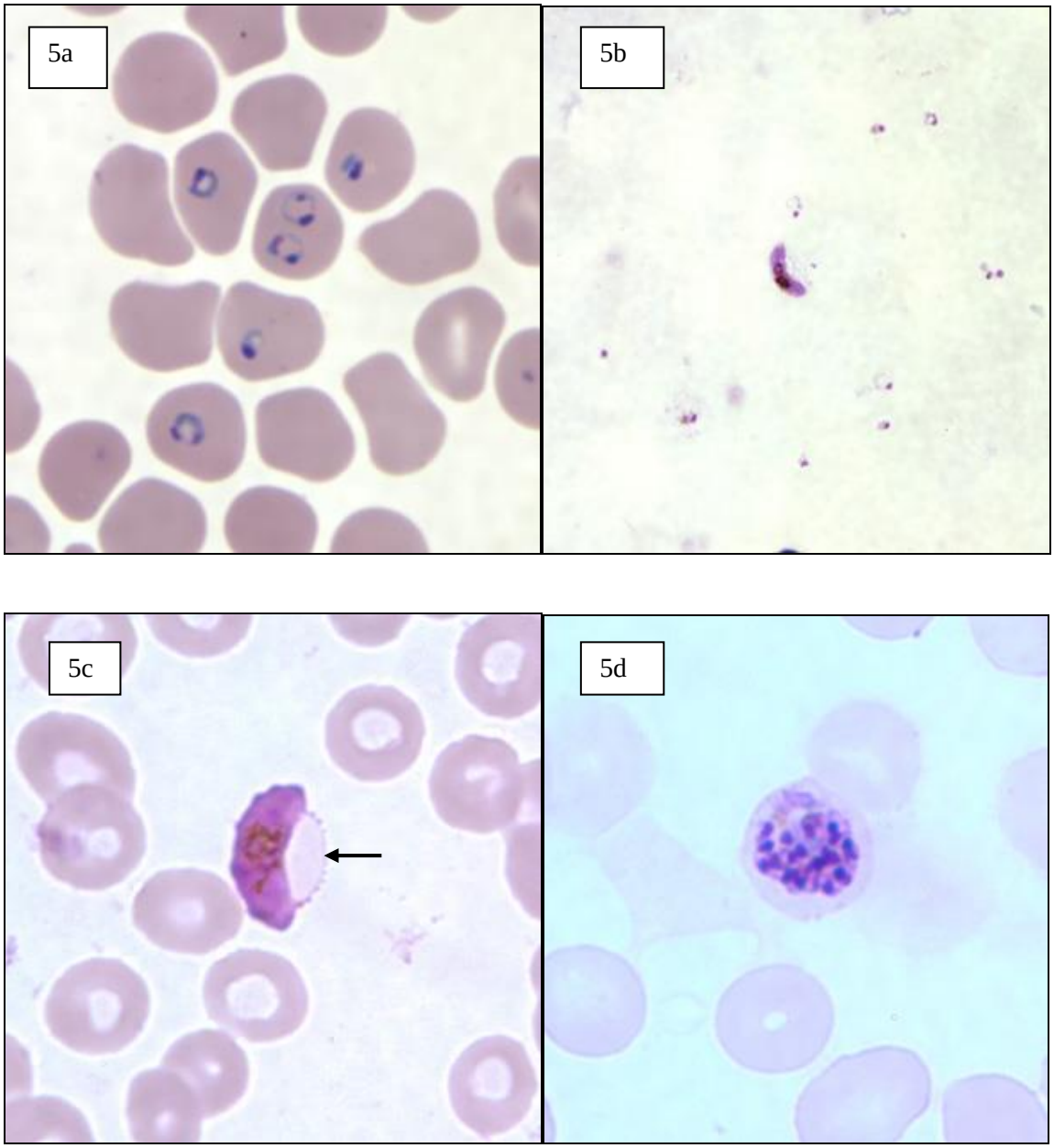


Figure 5 Microscopic appearance of stages of Falciparum

[Adapted from CDC – Malaria gallery]

4.4.2 RAPID DIAGNOSTIC KITS:

The following antigens have been incorporated as a part of rapid diagnostic tests. Currently the antigens which are in use include: histidine rich protein(HRP2),plasmodium lactate dehydrogenase(Pldh) and aldolase (18).These tests are of use in resource limited endemic

countries like sub Saharan africa,where diagnosis by standard microscopy is time consuming with lack of expertise.

4.3 MANAGEMENT OF MALARIA

Principles of treatment include the use of combination therapy to prevent the parasite from acquiring resistance. This may occur following exposure to single pharmacological agent, and to retain the efficiency of the antimalarials.The mechanism of action of antimalarials are summarised in Figure 6.

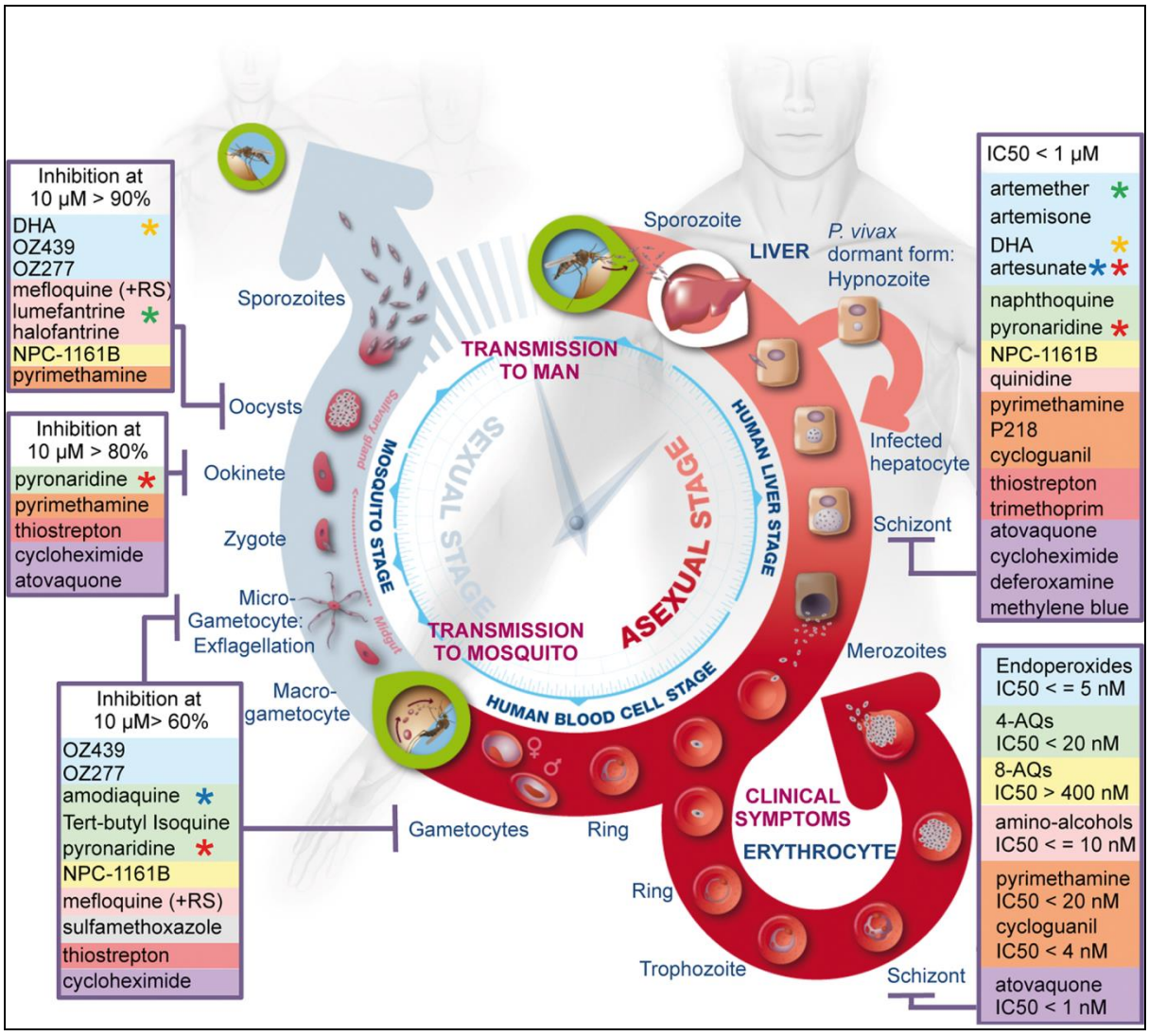


Figure 6 Mechanism of Antimalarials(19)

4.3.1 UNCOMPLICATED FALCIPARUM MALARIA:

Uncomplicated malaria is defined as malarial infection with parasite index less than 5 percent with absent organ involvement and ability to take oral therapy.

Treatment is guided based on the resistance patterns to chloroquine.

4.3.1.1 In chloroquine sensitive areas:

This is mainly mid east, Central America, Haiti and Dominican Republic countries. Chloroquine is safely used as first line treatment. The dose is summarised as follows:

600 mg oral base at diagnosis followed by 300 mg .The dose should be repeated at 6, 24 and 48 hours.

4.3.1.2 In chloroquine resistant areas:

ACT are the recommended first line antimalarials for treatment of *falciparum* malaria(20).ACT are active against all developmental stages of the asexual forms of malaria which leads to rapid clearance time compared to other agents. ACTs have demonstrable activity against gametocytes; however it does not target mature forms. The following treatment regimens are recommended:

4.3.1.2.1 Artemether + lumefantrine:

It is given as combination tablets (1 tablet = 20 mg artemether and 120 mg lumefantrine). A total of 6 oral doses are recommended based on weight (25 - <35 kg: 3 tablets per dose, ≥35 kg: 4 tablets per dose). The initial dose should be followed by the second dose which is after 8 hours followed by 1 dose twice daily for the following two days, for a duration of 3 days.

4.3.1.2.2 Artesunate + amodiaquine

This is distributed as tablets containing 50 mg of artesunate and 153 mg base of amodiaquine. The recommended dose is 4 mg/kg of artesunate and 10 mg/kg of amodiaquine given once a day for three days.

4.3.1.2.3 Artesunate + mefloquine

This is given as separate tablets containing 50 mg of artesunate and 250 mg base of mefloquine, divided over 2 or 3 days to facilitate absorption. The recommended treatment is 4 mg/kg of artesunate given once a day for three days and 25 mg base/kg of mefloquine.

4.3.1.2.4 Artesunate + sulfadoxine-pyrimethamine

This combination is distributed as 50 mg of artesunate, and tablets containing 500 mg of sulfadoxine with 25 mg of pyrimethamine. The total recommended treatment is 4 mg/kg of artesunate given once a day for three days and a single administration of sulfadoxine-pyrimethamine (25/1.25mg base/kg) on day 1.

4.3.1.2.5 Dihydroartemisinin+ piperaquine

The 1st, 2nd and the 4 th combinations are commonly used in India. In a metaanalysis of the available 16 randomised controlled trials comparing artesunate to conventional treatment in *falciparum* malaria, artemisinin was found to hasten parasite clearance and decrease transmission potential(21).It was also found to decrease treatment failure rates and recrudescence.

4.4 MANAGEMENT OF SEVERE MALARIA

Severe malaria is defined as malarial infection with presence of organ dysfunction or parasite index more than 5%.

The organ involvement can be listed as follows:

1) Metabolic acidosis

2) Circulatory collapse/shock

3) Acute respiratory distress syndrome/pulmonary edema

4) Haemoglobinuria

5) Anaemia

6) Thrombocytopenia

7) Acute kidney injury

8) Acute liver failure/hepatic encephalopathy

9) Hypoglycaemia

10) Altered sensorium/seizures/cerebral malaria

Presence of any one of the above mentioned organ dysfunction, is severe malaria.

Severe malaria is managed by parenteral anti malarial mainly quinine or artemisinin based combination therapy.

4.5 EFFICACY OF ARTEMISININ

The landmark trial ‘AQUAMAT’ comparing quinine and artesunate in management of severe malaria in African children, showed favourable results for artesunate with 22.5% absolute risk reduction of mortality between groups, which was the primary outcome(22). Tolerance to artesunate was much better with no significant adverse effects when compared to quinine.

The trial conducted in South east Asian population, recruiting 730 patients from India, Myanmar, Indonesia and Bangladesh ‘SEAQUAMAT’ showed a 34.7% absolute reduction in the mortality in the artesunate group as compared to the quinine(23).

Current evidence favouring artemisinin over quinine is from the published metanalysis in 2012,which included 8 major randomised control trials including ‘Aquamat’,’Seaquamat’ comprising of 1664 adults and 5765 children(24).In adults the relative risk for the outcome of death was found to be 0.64 over parenteral quinine and was found to be of statistical significance(24).The evidence was used by the World Health Organisation in 2010 to formulate the treatment guidelines in 2010,where parenteral artesunate is recommended as first line management of severe malaria(20).

The recommended standard dosing regimen includes a 5 dose method which is 2.4 mg/kg at admission, 12 hours and 24 hours followed by once dosing everyday. Complications are to be managed appropriately. Therapy can be changed to oral once the patient tolerates or general condition shows an improving trend.

4.6 PHARMACOLOGY OF ARTEMISININ COMPOUNDS

4.6.1 HISTORY OF ARTEMISININ COMPOUNDS:

These compounds are derivatives of natural plant product, from the plant *Artemisia annua* and was discovered as early as 168 B.C(25).This was extracted and purified and was developed further as a drug by Chinese scientists. The potency was confirmed by animal studies and was demonstrable in nano molar concentrations.

4.6.2 MECHANISM OF ACTION:

Artemisinin belong to the endoperoxides,which is a potent antimalarial agent .They are classified as first and second generation compounds(26).The first generation is comprised of the natural product artemisinin as well as multiple other semi synthetic derivatives like artesunate and artelinate which are water soluble ,arteether and artemether are fat soluble derivatives .They belong to a broad class of sesquiterpene lactones, which contain the unique endoperoxide bridge, which is believed to cause the parasite destruction. The endoperoxide compound activates the formation of reactive oxygen species, which surpasses the parasite anti oxidant system and the intra erythrocytic free radicals produced by the host , leading to death of the parasite(27).

The second postulated mechanism involves decomposition of the artemisinin compound in the presence of ferrous ion to from carbon centred free radical which reacts biochemically with the parasite and causes destruction(28).

It has also been hypothesized that the artemisinin compounds form adducts with haemozoin present in the food vacuole of the parasite, which further leads to activation of artemisinin as a potent alkylating agent(29).This has been found to be augmented with addition of iron chelators like deferrioxamine.

The molecular target for artemisinin is SERCA – Sarcoendoplasmic reticulum calcium ATPase located in the pfATP6 locus of the Falciparum genome(30).After the activation of the compound by iron, the compound inhibits the pfATP6 which is outside the food vacuole, specifically the SERCA sequence, leading to parasite destruction.(30).

4.6.3 PHARMACOKINETICS OF ARTEMISININ DERIVATIVES:

Derivatives of artemisinin commonly used in clinical practice include:

-Artesunate

-Artemether

-Dihydroartemisinin (DHA)

The summary of the published data on pharmacokinetic parameters of artesunate and DHA post intravenous administration(31) are summarised in table 1 and 2.

4.6.3.1 ARTESUNATE PHARMACOKINETICS

The metabolism of artesunate occurs by esterase catalysed hydrolysis causing the formation of dihydroartemisinin, its potent metabolite. This process occurs very rapidly leading to rapid depletion of artesunate in the immediate post dose period. The average half life of intra venous artesunate in the published studies was less than 15 minutes(31).It was also seen that there was liner dose response relation in doses from 0.8 mg/kg to 8 mg /kg(31).

EVIDENCE ON PHARMACOKINETICS OF IV ARTESUNATE

REFERENCE	SUBJECTS	DOSE	CMAX	AUC
Batty le et al,1998(32)	12 adults with *vivax* malaria from Vietnam	120 mg IV AS over 2 min	13685	876
Batty,Thu et al,1998(33)	26 adult uncomplicated *falciparum* malaria patients in Vietnam	120 mg IV AS over 2 min	NA	1146
Ilett et al,2002(34)	23 adults with uncomplicated *falciparum* Malaria in Vietnam	120 mg IV AS over 2 min	16146; 16530	1038; 1230
Newton et al,2006(35)	17 adults with severe *falciparum* malaria in Thailand	2.4 mg/kg IV AS over 2 min	130	49.2
Li et al,2009(36)	30 healthy volunteers – phase I study	0.5 mg/kg IV AS over 2 mins	4797	386
		1 mg/kg IV AS over 2 mins	6128	593
		2 mg/kg IV AS over 2 mins	19420	1595
		4 mg/kg IV AS over 2 mins	36100	3038
		8 mg/kg IV AS over 2 mins	83340	699
Binh et al,2010(37)	17 healthy volunteers from Vietnam	120 mg IV AS over 2 min	NA	846; 1269

Table 1 – ARTESUNATE PHARMACOKINETICS [Adapted from Morris et al,Malaria Journal,2011]*(31)*

4.6.3.2 DIHYROARTEMISININ PHARMACOKINETICS:

The metabolism of dihydroartemisinin is through the UDP glucoronyltransferase pathway, in particular UGT1A9 and UGT2B7(38).The esterase catalysed process of metabolism occurs slowly as compared to artesunate(31).The average half life as documented in the published

studies range from 18 minutes to 2.14 hours. Similar dose linearity as expressed by artesunate is also seen with dihydroartemisinin(31).

EVIDENCE ON DIHYDROARTEMISININ PHARMACOKINETICS				
REFERENCE	SUBJECTS	DOSE	CMAX	AUC
Batty le et al,1998(32)	12 adults with *vivax* malaria in Vietnam	120 mg IV AS over 2 min	2192	1845
Batty Thu et al,1998(33)	26 adult uncomplicated *falciparum* malaria patients in Vietnam	120 mg IV AS over 2 min	2648	2377
Binh et al,2001(37)	17 healthy volunteers from Vietnam.	120 mg IV AS over 2 min	1507	NA
Davis et al, 2001(39)	30 adults with falciparum malaria 1: 12 with complications 2: 8 without complications	Group 1 & 2: 120 mg IV AS over 2 min Group 3: 10 with moderately severe complications (240 mg IV AS infused over 4 hours	Group 1: 2417 Group 2: 2531 Group 3: 910	Group 1: 2078 Group 2: 2559 Group 3: 5573
Ilett et al,2002(34)	23 adults with *falciparum* malaria with no complications from Vietnam	120 mg IV AS over 2 min	2758,2730	2872,3298
Newton et al,2006(35)	17 adults with severe *falciparum* malaria in Thailand	2.4 mg/kg IV AS over 2 min	605	418

Table 2 DIHYDROARTEMISININ PHARMACOKINETICS[Adapted from Morris et al, Malaria journal 2011]*(31)*

It has been proved without doubt that the maximum concentrations of artesunate is achieved post intravenous administration, hence it's utility in emergent management of severe malaria.

4.6.4 EFFECT OF INFECTION STATUS ON PHARMACOKINETICS:

Population based pharmacokinetic studies have shown that the AUC and the peak concentrations of the drug level differ based on the severity and type of infection. Apart from the presence of food(for oral administration) and body weight, clearance of DHA was found to be lower on the first day of malaria when compared to day 3 of illness(40).Evidence also shows that the peak antimalarial activity is twice higher in the acute phase than the convalescent phase(41).But these were done on oral formulations and scarce data exists for intravenous formulations.

4.7 HISTORY OF ANTIMALARIAL RESISTANCE

The earliest evidence of anti malarial resistance dates back to the early 20 th century when resistance to chloroquine emerged in the 'Greater Mekong' region, which was soon followed by sulphadoxine – pyrimethamine and mefloquine(42)

4.8 ARTEMISININ RESISTANCE

The first report of suspected resistance through delayed parasite clearance times was communicated to the world by Noedl et al(6) in the year 2008,which prompted further studies which provided an insight to this threat. Subsequent evidence by Dondorp et al(5) alerted the World Heath Organisation(WHO) to formulate and standardise case definitions to conduct therapeutic surveillance studies.

Routine therapeutic surveillance is essential in malaria endemic regions to determine the parasite susceptibility patterns, and if required to revise the treatment policies based on the local sensitivity patterns. It is currently recommended by WHO, that regular studies be done once in 2 years, and if failure rates exceed 10% at day 28/day 42 of follow up, the treatment regimen has to be revised.

4.8.1 DEFINITION(43)

The definition of artemisinin resistance depends on clinical and parasitological outcomes observed during routine therapeutic efficacy studies of ACTs and clinical trials of artesunate monotherapy:

- an increase in parasite clearance time, as evidenced by ≥ 10% of cases with parasites detectable on day 3 after treatment with an ACT (suspected resistance)

(Or)

- treatment failure after treatment with an oral artemisinin-based monotherapy with adequate antimalarial blood concentration, as evidenced by the persistence of parasites for 7 days, or parasites being detected at day 3 and recrudescence within 28/42 days (confirmed resistance)

The latest development in this field is the identification of the novel mutation "kelch -13" which is found to be signatory molecular marker of artemisinin resistance. Hence the definitions have been modified, taking this into consideration and the following has been proposed in the September 2014 update by WHO.

4.8.2 SEPTEMBER 2014 UPDATE ON DEFINITION OF PARTIAL ARTEMISININ RESISTANCE(44)

Suspected partial artemisinin resistance is defined as:

- ≥ 5% of patients carrying K13 resistance associated mutations

(Or)

- ≥ 10% of patients with persistent parasitemia after treatment with ACT /monotherapy with artesunate detected by microscopy on day 3.

(Or)

- ≥ 10% of patients with a parasite clearance half life of ≥ 5 hours after

treatment with ACT or artesunate monotherapy.

Confirmed partial artemisinin resistance is defined as:

≥ 5% of patients with detectable Kelch 13 mutations, and other resistance associated mutations after treatment with ACT or artesunate monotherapy, to have either persistent parasitaemia by microscopy on day 3, or a parasite clearance half life of ≥ 5 hours.

4.8.3 CLINICAL IMPLICATIONS OF DELAYED PARASITE CLEARANCE

1) Failure to clear the parasites rapidly will compromise the care of patient in severe malaria

2) It leads to prolonged exposure of the parasite to the partner drug leading to development of resistance to partner drug.

4.8.4 EMERGENCE AND SPREAD OF RESISTANCE TO ANTIMALARIAL DRUGS

The resistance of parasite develops as two parts:

In the first part, an initial genetic event produces a resistant mutant (de novo mutation), the genetic alteration gives the parasite a survival advantage against the drug.

In the second part, the resistant parasites are selected and they replicate resulting in a parasite population that is no longer susceptible to treatment.

4.8.5 FACTORS INFLUENCING DEVELOPMENT OF ANTIMALARIAL RESISTANCE

- The frequency of genetic mutations.
- The degree of resistance caused by the mutations
- The resistance mechanism
- The proportion of gametocytes/vectors exposed to the drug (selection pressure)
- The exposed number of parasites to the drug
- The concentration of the anti malarial drug to which the parasites are exposed

- The pharmacogenomics of the drug - Individual dosing, duration, adherence – the variation and use in the community including the quality, availability, distribution and patterns of drug use
- The profile of the innate and acquired immunity of the person as well as the community
- The presence of detectable drugs in blood to which the parasite is sensitive

Poor quality of drugs is another important factor of major concern in South East Asia. Newton et al conducted an epidemiological investigation to identify the prevalence of fake artesunate in South east Asia(45).The study revealed 16 fake stickers on packages of the drugs manufactured, falsely identifying them as genuine. Chemical analysis revealed undetectable or extremely low levels of artesunate in those samples. There was significant adulteration with compounds such as paracetamol, older anti malarial drugs, minerals and antibiotics. This became further more apparent when a Burmese young gentleman succumbed to malaria with a PI of 5% despite having received the optimal dose of artesunate(46).

India being a developing nation predisposes to unauthorised trades of pharmaceuticals across borders and distribution in areas with high endemicity, posing a serious threat of emergence of resistance secondary to exposure to sub therapeutic concentrations of artesunate.

4.8.6 MONITORING ANTI MALARIAL DRUG EFFICACY AND DRUG RESISTANCE

There are four major methods to assess anti malarial drug efficacy and to monitor emergence of resistance(47).

1) Therapeutic efficacy studies
2) In vitro tests
3) Use of molecular markers
4) Measurement of drug concentrations

Therapeutic efficacy studies are the current gold standard for determining antimalarial efficacy(4).

4.8.7 GLOBAL PLAN FOR ARTEMISININ RESISTANCE CONTAINMENT

The GPARC was established in response to confirmation of artemisinin-resistance in Cambodia and Thailand(5).This was initiated as a response to the emergence of resistance and to contain the spread of drug resistance malaria.

The GPARC defined three areas of artemisinin resistance:

TIER I – areas where confirmed evidence of artemisinin resistance has been documented, where an immediate, team based response is recommended to contain or eliminate resistant parasites.

TIER II - areas with movement of mobile and migrant populations from tier I areas or shared borders with tier I areas, with strict and vigilant malaria control to reduce transmission and/or limit the risk of emergence or spread of resistant parasites.

TIER III - *P. falciparum* endemic areas where there is no evidence of artemisinin resistance and have the contact with tier I areas is limited, where prevention and increasing coverage with parasitological diagnostic testing, quality-assured ACTs and vector control is required.

4.8.8 SUMMARY OF ARTEMISININ RESISTANCE IN GREATER MEKONG REGION

The pioneer trial which identified prolonged fever and parasite clearance times in South east asia,mainly in Pailin of western Cambodia and the north western Thai Cambodian Border published by Dondorp et al(5),attracted the world's attention, to the serious threat of resistant malaria. Since then containment efforts and research are underway to stop the spread of resistance as well as to work towards elimination of the disease.

Currently the Greater Mekong region is the epicentre of artemisinin resistance. The proportions of patients with detectable parasitemia on microscopy at 72 hours (day 3), is the early sign to indicate emergence of artemisinin resistance(48).

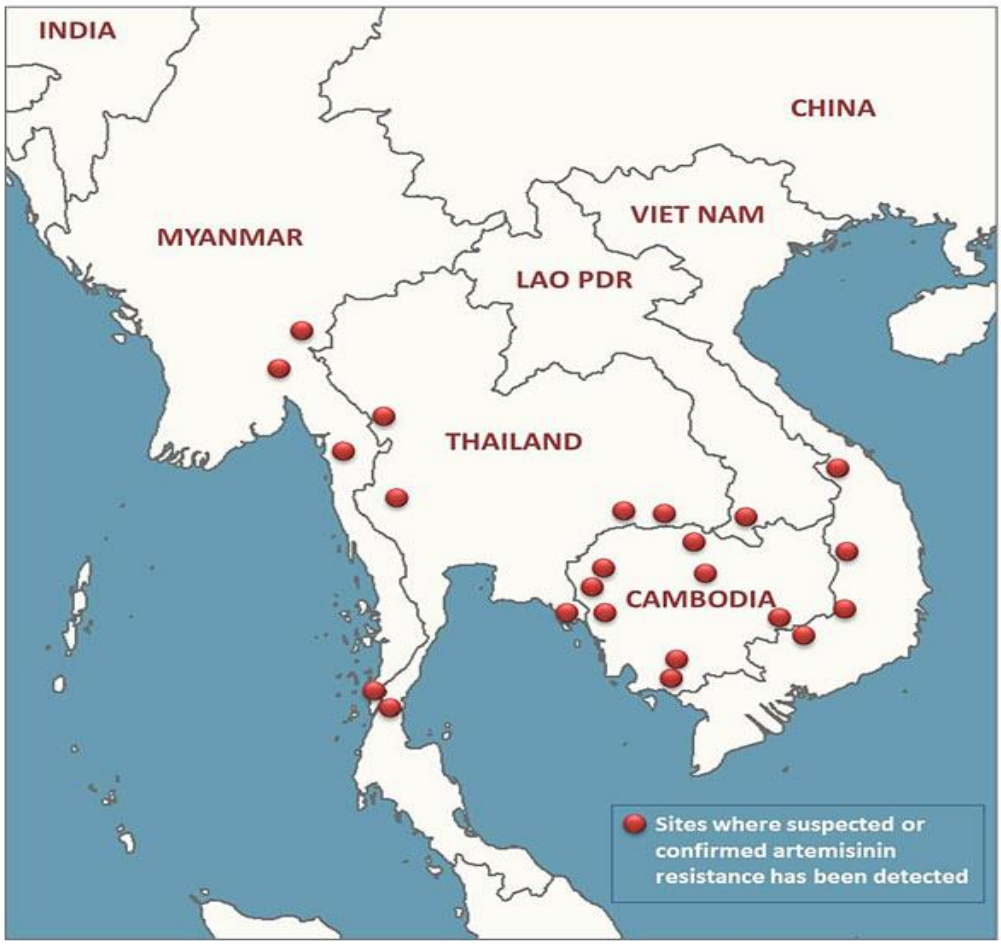


Figure 7 Sites of confirmed resistance to artemisinin

[Adapted from 'Emergency response to artemisinin resistance in Greater Mekong Sub region WHO 2013]*(49)*

The summary of data on day 3 parasitemia rates and treatment failure rates have been summarised in the table:

Summary of the status of artemisinin resistance in the Greater Mekong Subregion

	artemisinin resistance		containment activities started	AL		AS-MQ		DHA-PPQ	
	suspected year of emergence	detected		D3+	TF	D3+	TF	D3+	TF
Cambodia	2001*	2006	2009	◆	◆	◆	◆	◆	◆
Laos	2013	2013	2014	◆	–				
Myanmar	2001*	2008	2011	◆	–	◆	–	◆	–
Thailand	2001*	2008	2009	◆	◆	◆	◆		
Viet Nam	2009	2009	2011					◆	–

Legend: first-line treatment; * detected retrospectively using molecular markers or retrospective data; ◆ observed to be > 10%; – observed to be < 10%; blank = undetermined

Figure 8 Status report on artemisinin resistance 2014

4.8.9 SPREAD OF ARTEMISININ RESISTANCE

An international multicentre randomised controlled trial was conducted at 15 sites in 10 countries, including 4 sites in Cambodia,3 sites in Thailand, Laos, Vietnam, Myanmar, Bangladesh, India,Nigeria,Kenya and Democratic republic of Congo(50).

Prolonged parasite clearance was seen in western Cambodia and eastern Thailand with parasite clearance half-life more than 5 hours (49 to 73%).The same was noted to be 14 to 28% in northern Cambodia, Vietnam, and eastern Myanmar, and very low proportions in other centres.

Resistance is now viewed as sequelae of widespread use of this drug and the rates of spread are accentuated by the socio economic conditions in different geographic regions(51).

The way to curb the spread of resistance is to conduct more surveillance in areas where the resistance rates are unknown. Second major intervention is to discourage artesunate monotherapy, which is a prevalent practice in private sectors in South East Asian countries.

4.8.10 KELCH 13 PROPELLER POLYMORPHISM – SIGNIFICANCE

A molecular marker has been identified to monitor the spread of resistance ,which is a sequelae of mutations in portions of *P.falciparum* gene (*PF3D7_1343700*) encoding Kelch (K13) propeller domains(52,53).A team of international scientists produced an artemisinin-resistant parasite line by exposing the artemisinin sensitive parasite to incremental doses of artemisinin over a period of 5 years. This developed resistant clone was whole-genome sequenced with comparison to the parental sensitive clone.

Seven new genes containing eight mutated single nucleotide polymorphisms were identified in the resistant clone. These mutations were compared with the genotypes found in Cambodian – Thailand borders, which to further identification of mutations in the propeller domain of the kelch protein K13.This is the signatory molecular maker of artemisinin resistance.

This will help in the characterization of artemisinin resistance at molecular level and also assists in monitoring of the spread of artemisinin resistance in the community. The *P. falciparum* cysteine protease falcipain-2 (FP2; *PF3D7_1115700*) has also been shown to contribute to mechanism of action of artemisinin. A stop mutation has also been identified in the FP2 gene which was subsequently noted on sequencing the parasites in patients with delayed clearance(53).

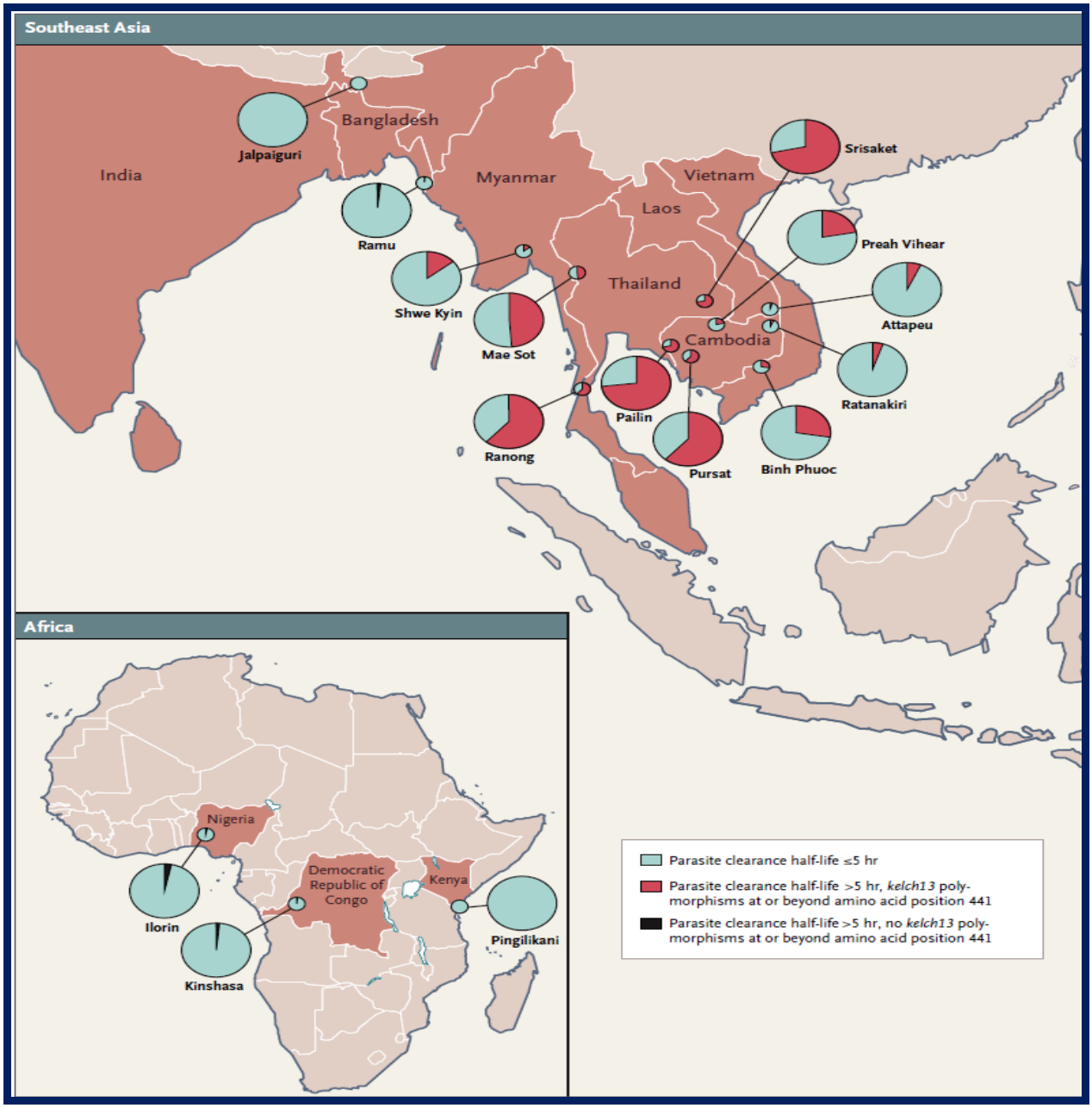


Figure 9 Current global scenario of artemisinin resistance

[Adapted from The New England Journal of Medicine(7)]

5. METHODS

5.1 SAMPLE AND SETTING

The study was conducted between October 2012 and June 2014 at CMC, Vellore. Patients presenting with acute febrile illness, diagnosed to be malaria, fulfilling the inclusion criteria and willing to participate in the study were included into the study. This included hospitalised patients with malaria as well as patients attending the emergency department and the General medicine outpatient clinic. The study and the research procedures were fully explained to the participants and those who gave written consent were allowed to participate in the study. The consent was obtained in the regional language that the patient/relative was conversant (Annexure 1)

5.2 STUDY DESIGN

This is a prospective cohort study done in patients with malaria, assessing the treatment response to artemisinin combination therapy.

5.3 SAMPLE SIZE

The sample size required to show an incidence of treatment failure to artemisinin combination therapy as 25% with 10 % precision was calculated by using the formula:

$4pq/d^2$.This was found to be 75.

5.4 PARTICIPANTS

The participants in this study fulfilled the following inclusion criteria:

1) Age between 15 years to 60 years;

2) *P. Falciparum* infection alone or a mixed infection as detected by microscopy

3) Detectable asexual forms in peripheral smear with minimal detectable parasite index of 0.1%

4) Presence of axillary temperature ≥ 37.5 °C or oral or rectal temperature of ≥ 38 °C or history of fever during the past 24 hours.

Presence of causes other than malaria for the fever, were excluded.

5.5 MEASUREMENTS – DATA COLLECTION

The data collection was done in data abstraction forms (Annexure 2) by the principal investigator of the study at the first visit, day 2, day 3 and day 7.

The following details were recorded specifically:

1) Demographics – Age, sex, geographic location, occupation and history of travel to malaria endemic area.

2) Duration of symptoms

3) Clinical assessment details – vital signs, systemic examination

4) Presence and duration of complications

5) Laboratory parameters including haematological and biochemical tests.

5.5.1 BODY TEMPERATURE

Axillary temperature was measured at baseline (day 0 before dosing) and on days 1, 2, 3 and day 7 or till discharge whichever was earlier till defervescence.

Temperature was measured with a thermometer that has a precision of 0.1 °C. Temperature was measured as clinically indicated. If the result is < 36.0 °C, the measurement was repeated. The same route was used throughout the study.

In outpatients, they were advised to manually record their temperature (under supervision by an health care professional), till defervescence.

They were followed up every day till first 3 days and on day 7 with temperature charting.

5.5.2 MICROSCOPIC BLOOD EXAMINATION FOR MALARIAL PARASITES

Blood samples for malarial parasites were collected twice daily from the patients, till the clearance of asexual ring forms from peripheral smears.

The peripheral blood was centrifuged to obtain the quantitative Buffy coat (QBC) and was examined for the presence of malarial parasites. If present, thick and thin smears were done in the sample to identify the species in order to confirm adherence to the inclusion criteria.

Parasite density was recorded in terms of parasite index. The parasite index was calculated in the smear by counting the number of parasitized red blood cells per 1000 red blood cells. This procedure was repeated every 12 hourly till disappearance of asexual ring forms in the QBC.The minimum value of parasite index was 0.1%.

5.5.3 ANTI MALARIAL ARTESUNATE – DIHYDROARTEMISININ BLOOD CONCENTRATION

Anti malarial blood concentrations were measured in patients on intravenous artesunate for severe malaria. Hospitalised consecutive patients who fulfilled the following inclusion criteria were included for the pharmacokinetic assessment studies of artesunate.

- Hospitalised patients on Intravenous artesunate.
- The timing of dose 3 is in the normal course after obtaining the results of the peripheral smear.
- Patients who fulfil the criteria for early treatment failure (asexual ring stages positive on day 3) and those having adequate therapeutic response were included for pharmacokinetic assessment.
- Willingness to participate in the study

The samples were collected by the principal investigator in the clinical pharmacology unit.

The patients were fasting prior to the procedure, only sips of fluid were allowed prior to the procedure.

A 23 gauge insyte (peripheral venous line) was inserted into the cubital vein of the opposite arm to which the IV drug (Artesunate) was administered.

Venous blood specimens of 2 ml each was collected at trough, 5, 7, 9,15, 30, 45, 60, 90 min, 120 and 240 minutes post dosing with intravenous artesunate.

5.5.3.1 DEVELOPMENT OF DRUG ASSAY – ARTESUNATE AND DIHYDRO ARTEMISININ

The instrument used for the assay – 'ACQUITY UP/LC – MS/MS'

COLUMN - Phenomenex Luna 5u PFP, diameter 50* 2 mm

MOBILE PHASE – A – 2 m mol ammonium acetate buffer in water + 0.1% formic acid

B – Acetonitrile + 0.1 % formic acid

GRADIENT

Time(mins)	Flow(ml/min)	% A	% B	Curve
0	0.4	80	20	
0.5	0.4	30	70	6
2	0.4	80	20	11

The flow was stopped at 3 minutes.

COLUMN TEMPERATURE – 35 deg C

EXTRACTION PROCEDURE:

- 100 ml of plasma sample was taken.
- 400 micro litre of acetonitrile containing internal standard (Artemisinin-40 ng/ml) was taken.
- The vertex was set at 1 minute.
- This was centrifuged at 13000 rpm for 5 minutes.
- The supernatant was separated and 20 micro litre was injected into the column.

MRM FILE (MULTIPLE REACTION MONITORING)

Compound name	Parent mass	Daughter mass	Dwell time	Cone voltage	Collition voltage
Artesunate	402.24	267.29	0.1	15	10
Dihydroartemisinin	302.1	267.1	0.1	12	8
Internal standard(artemisinin)	300.08	209.4	0.1	13	12

MS TUNE PAGE

Capillary (kV) – 4

Cone (V) – 20

Extractor (v) – 2

RF Lens (v) – 0.4

Source temperature (deg C) – 100

Desolvation temperature (deg C) – 400

Desolvation gas flow (l/hr) – 800

Cone gas flow (l/hr) – 50

Collition gas flow (ml/min) – 0.16

Plasma concentrations of artesunate and dihydroartemisinin (the active metabolite of artesunate) were measured by means of high - throughput liquid chromatography -tandem mass spectrometry, (LC-MS/MS) in the Clinical Pharmacology Unit.

The AUC (Area under curve), and other pharmacokinetic parameters as Cmax (Maximum concentration), trough, clearance was calculated.

The pharmacokinetic parameters between the two categories of patients were compared for any significant statistical difference.

The dose response relationship and the clinical outcomes were correlated through logistic regression (uni and multivariate) analysis

5.6 SAMPLES FOR IDENTIFICATION OF MOLECULAR MARKERS

Samples were collected from the patients enrolled into the study (Minimum – 1 and maximum of 3).

They were allowed to stand and the blood clot and serum were separated.

All the samples are stored in the Wellcome laboratory at -90 deg C for future studies on identifying the genetic markers of artemisinin resistance.

6. OUTCOMES

The following parameters were specifically assessed in this study.

6.1 PRIMARY OUTCOMES

6.1.1 Parasite clearance time - as assessed by microscopy.

The parasite clearance time was defined as the time from the start of treatment until the first negative blood smear.

6.1.2 Fever clearance time – as assessed by clinical methods.

The fever clearance time was defined as the time taken for temperature to fall below 37˚C and remain there for at least 24 hours. In other words, it was the time to the first temperature reading of less than 37.5°C and the time to the start of the first 24-hour period during which the temperature remained below 37.5°C.

6.1.3Gametocytemia in patients - Proportion of patients with gametocytemia before, during and after treatment with artesunate, assessed at admission, on days 3, 7 stratified by presence of gametocytes at enrolment.

Treatment outcomes were classified based on WHO guidelines for surveillance of anti malarial efficacy(47)

6.2 Early treatment failure (ETF):

- Danger signs or severe malaria on day 1, 2 or 3, in the presence of parasitemia;
- Parasitemia on day 2 higher than on day 0, irrespective of axillary temperature;
- Parasitemia on day 3 with axillary temperature ≥ 37.5 °C;
- Parasitemia on day 3 ≥ 25% of count on day 0

6.3 Late clinical failure (LCF)

- Danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 28 (day 42) in patients who did not previously meet any of the criteria of early treatment failure;
- Presence of parasitaemia on any day between day 4 and day 28 (day 42) with axillary temperature ≥ 37.5 °C in patients who did not previously meet any of the criteria of early treatment failure.

6.4 Late parasitological failure

- Presence of parasitaemia on any day between day 7 and day 28 and axillary temperature < 37.5 ºC in patients who did not previously meet any of the criteria of early treatment failure or late clinical failure.

6.5 SECONDARY OUTCOMES:

1) Comparison of the pharmacokinetics of artesunate and the metabolite dihydroartemisinin in patients with and without treatment failure, following initiation of artemisinin combination therapy (ACT) and comparison with the clinical and parasitological outcomes.

7. DATA ANALYSIS AND STATISTICAL METHODS

Data entry was done by the principal investigator in Microsoft Excel Spreadsheet (Annexure 4).

The results were analysed using SPSS software version 16.

The parasite clearance and fever clearance times were plotted against time and were assessed using the Kaplan Meier methods and the median clearance times were obtained.

Data was analysed by the student t test, chi square test or Mann Whitney U test, Fisher's exact test based on the normality of distribution of the variables.

The pharmacokinetic studies were analysed manually and the AUC was calculated for 4 hours exposure using the trapezoidal rule. The trapezoidal rule consists of dividing the plasma concentration-time profile into several trapezoids and calculating the AUC by adding the area of these trapezoids. The statistical correlation was obtained through Wilcoxon signed rank test and Mann Whitney U tests as applicable.

Univariate analysis was done to identify the factors which might be associated with delayed fever and parasite clearance times. The factors identified to show association were included in the multivariate analysis and the logistic regression models, to derive the odds ratio and measure the statistical significance.

The factors with which showed a trend towards association were included in boot strap analysis to generalize the finding to the parent population in a scientific manner to derive the degree of association

8. FUNDING AND APPROVAL

8.1 Funding Source

A FLUID Research grant (Institutional grant) was approved for the purpose of this study. The funds were used for the development of the artesunate and dihydroartemisinin LC – MS/MS assay and for processing of the samples, procurement of reagents and the pure samples.

8.2 Institutional Research Board approval and ethical considerations

The research proposal was discussed by the Institutional Review Board in October 2012 and approval was obtained [IRB Min. No.8056 dated 06.11.2012].

There were no ethical issues related to this study**.** Institutional review board approval was obtained for the procedures for the pharmacokinetic studies, prior to the commencement of the study (Annexure 5**)**

9. RESULTS

The study was conducted prospectively over a period of 20 months (October 2012 – June 2014) in the Department of Medicine, in the inpatient wards, emergency department and the outpatient departments in Christian Medical College,Vellore,a tertiary care centre in South India.

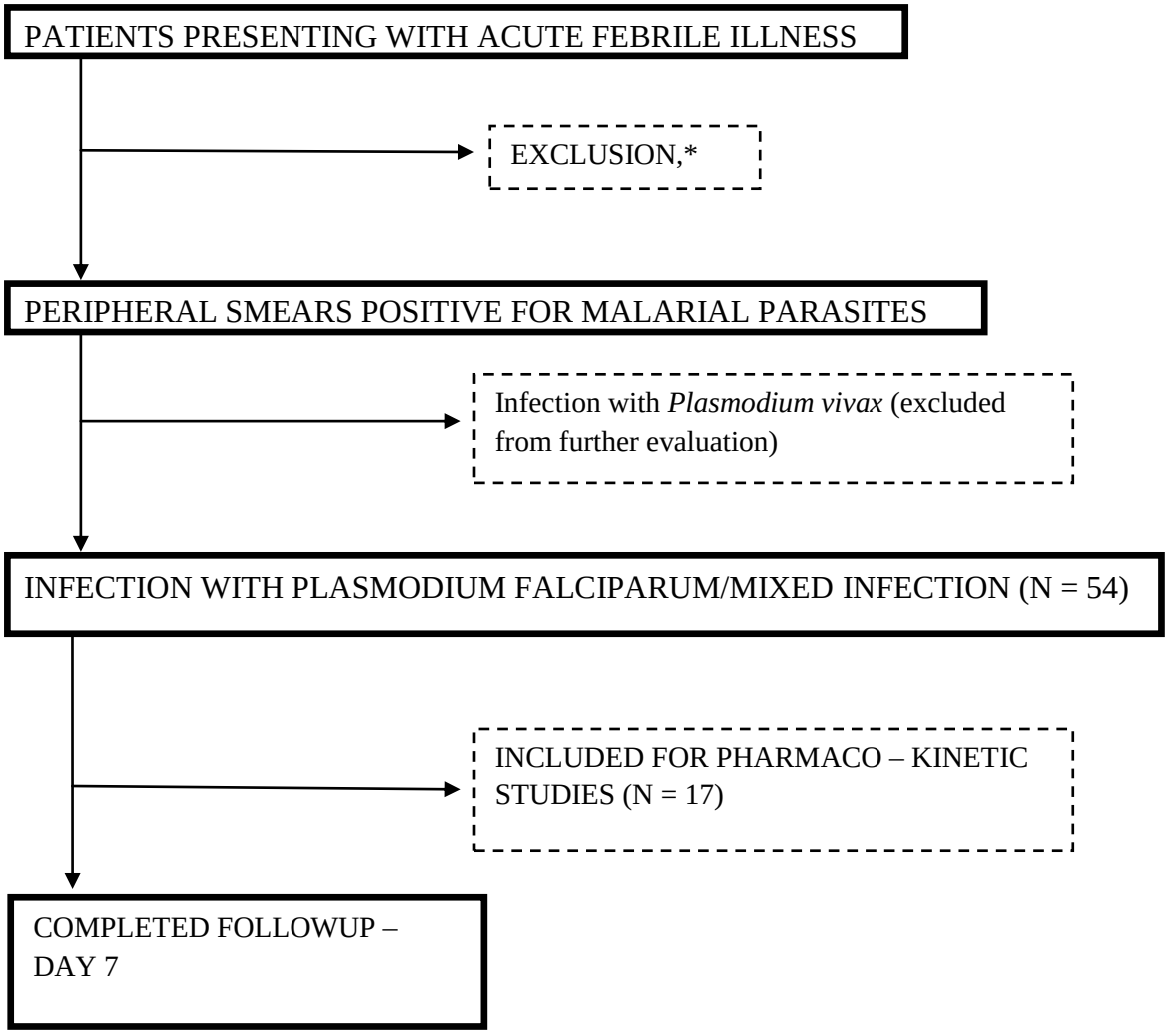
Clinical assessment included detailed history and physical examination was done on day 1, 2, 3 and 7 days. All the haematological and biochemical tests done and the parameters were noted in the Data abstraction forms (Annexure 5).

Patients with acute febrile illness, diagnosed as malaria, 15 years and above, who fulfilled the inclusion criteria were enrolled into the study.

Fifty four patients fulfilled the inclusion criteria and were included into the study.

All participants provided written consent to participate in the study, and 17 patients were enrolled into the pharmacokinetic studies of Intravenous artesunate following informed consent.

9.1 STUDY FLOWCHART



9.2 BASELINE CHARACTERISTICS OF THE POPULATION

9.2.1 DEMOGRAPHIC CHARACTERISTICS

A total of 54 adult patients more than 15 years of age, with peripheral smear positive for *Plasmodium falciparum* were evaluated.

The mean age (+_SD) was 37.59(14.147) with a range of 17 - 75 years.

The distribution of cases yearly and the time trends are summarised in Figure 10 and 11 .The number of cases were similar in 2012 and 2013,but the number of patients were distributed throughout the last 6 months in 2013,compared to 2012,when the peak was during October – December, corresponding to the monsoon season.

There was significant male preponderance (Figure 12). 46.3% were unskilled workers predominantly agricultural labourers followed by 25.9% being in the professional category, residing in places with malaria endemicity during the monsoons like Chennai (Figure 13).

The patients were mainly from the two states of Tamil nadu and Andhra Pradesh, with 57.4% in the former and 38.9% in the latter group.

Majority of the recruited patients were hospitalised inpatients (81.5%).

The history of travel to malaria endemic regions (Chennai, Chittoor and other pockets in Andhra Pradesh) was elicited in 37% of the patients.

33.3% had mixed malarial infection with *falciparum* and *vivax* species.

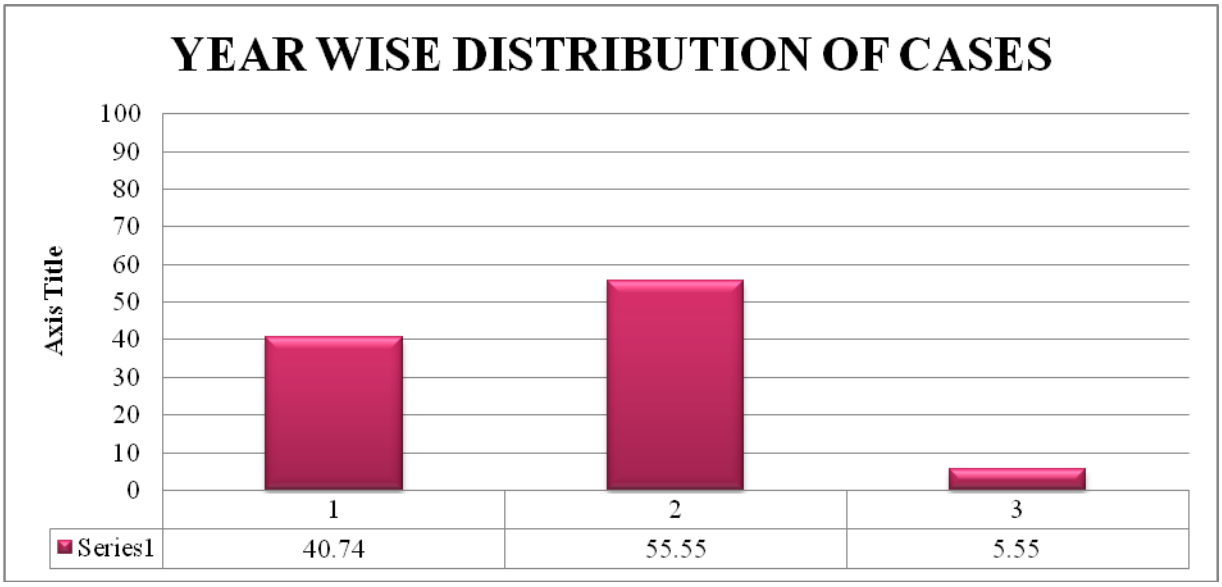


Figure 10 – Year wise distribution of malaria cases

1 -2012, 2 – 2013 ,3 - 2014

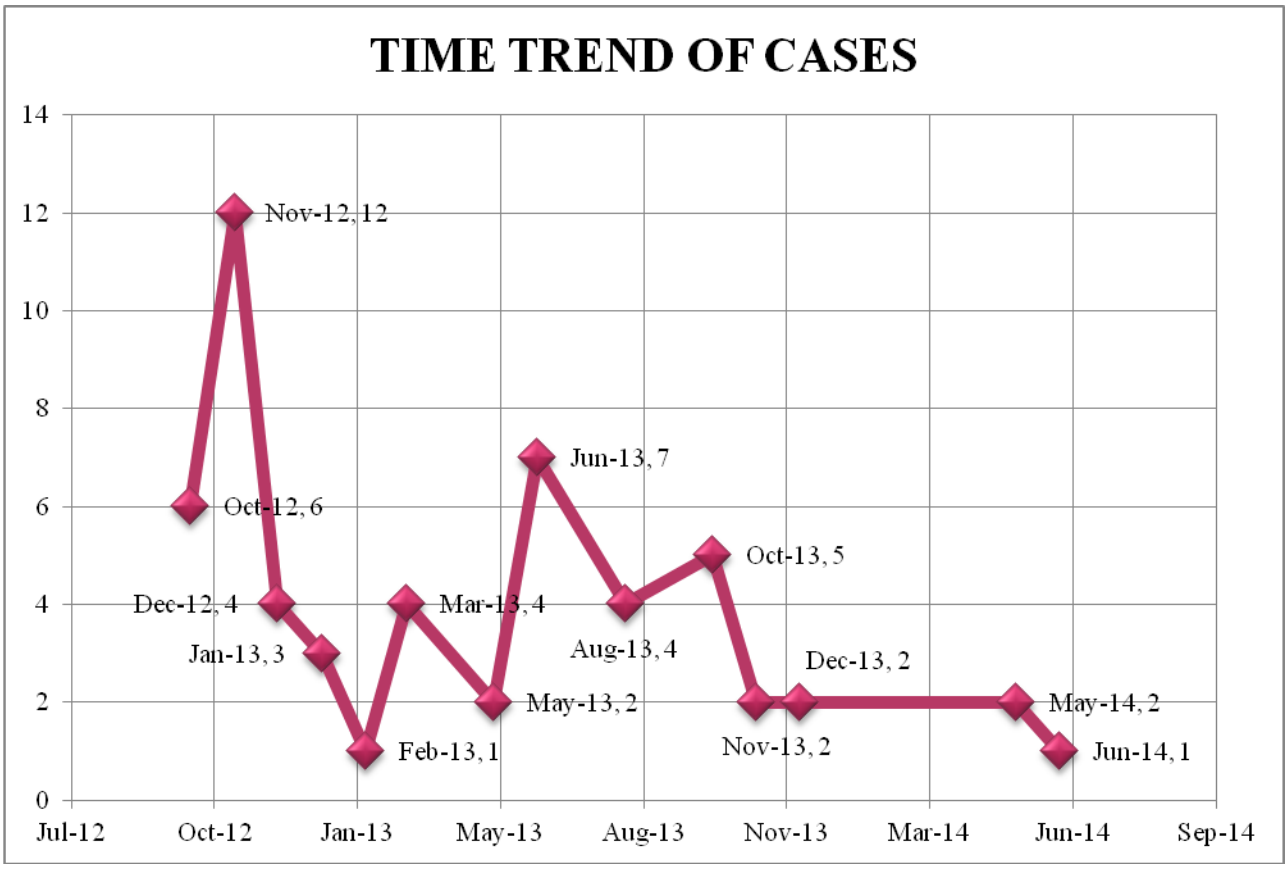


Figure 11 - Case distribution throughout the period of recruitment

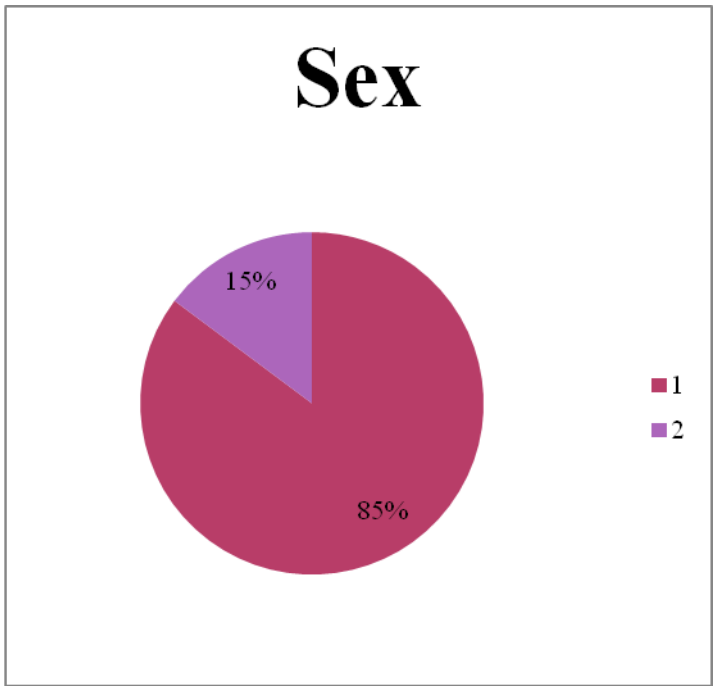


Figure 12- Sex distribution

(1 – Male, 2 – female)

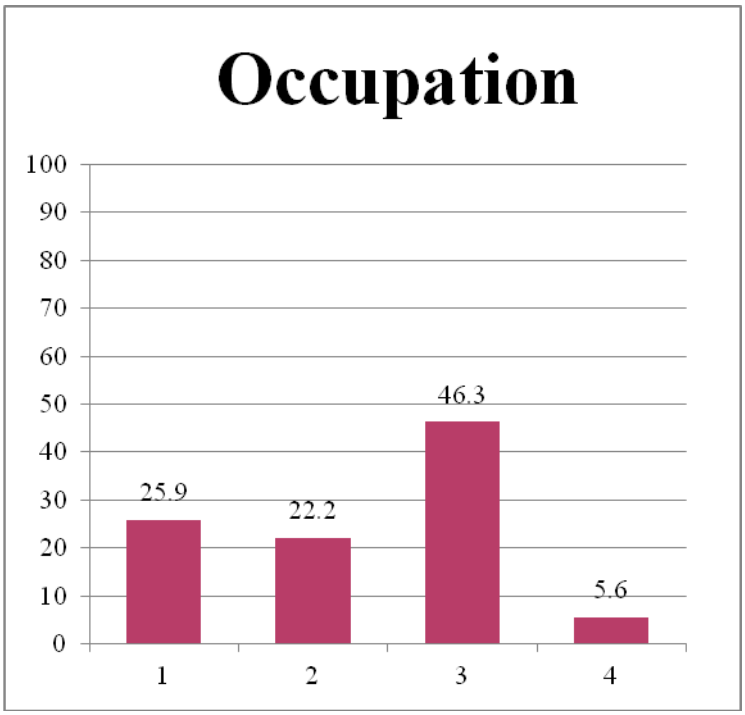


Figure 13 - Occupation

Professional(14)	1
skilled labourer(12)	2

Unskilled(25)	3
Unemployed(3)	4

9.2.2 PROFILE OF COMORBID ILLNESS

The percentage of co morbid illness was low, probably in view of the low mean age of the cohort, details are summarised in Table 3.

Variable	Number	%	Comments
Hypertension			
Present	3	5.6	
Absent	51	94.4	
Chronic Kidney Disease	0	0	
Present	54	100	
Absent			
CerebroVascular Accident	0	0	
Present	54	100	
Absent			
Chronic Liver Disease	1	1.9	**One patient had Hepatitis B related chronic liver disease**
Present	53	98.1	
Absent			
Diabetes Mellitus			**The median age of the diabetics were 46(40 – 56)**
Present	4	7.4	
Absent	50	92.6	
Ischemic Heart Disease	3	5.6	**All of the three patients had underlying diabetes and hypertension**
Present	51	94.4	
Absent			
HIV Co – infection			
Present	0	0	
Absent	54	100	
Chronic Obstructive Pulmonary disease			
Present	**0**	**0**	
Absent	**54**	**100**	

Table 3 Profile of co morbid illnesses

9.2.3 SUMMARY OF CLINICAL SYMPTOMS

The most common presenting symptom was fever which was present in all the patients. The median duration of fever prior to presentation was 7 days with minimum of 4 days to maximum of 60 days. 61.1% reported jaundice as defined yellowish discolouration of skin, mucous membranes or high coloured urine. 27.78% reported breathing difficulty or chest tightness at presentation .Altered sensorium was present in 24.07% which included drowsiness, decreased verbalisation or irrelevant speech, inadequate response to call or commands. 2 patients had seizures at presentation, both of which were generalised tonic clonic convulsions requiring anti epileptic therapy. Of the bleeding manifestations, 6 patients reported haematuria, 4 patients had haematemesis suggestive of upper GI bleed and 2 patients reported epistaxis.The findings are summarised in Figure

14

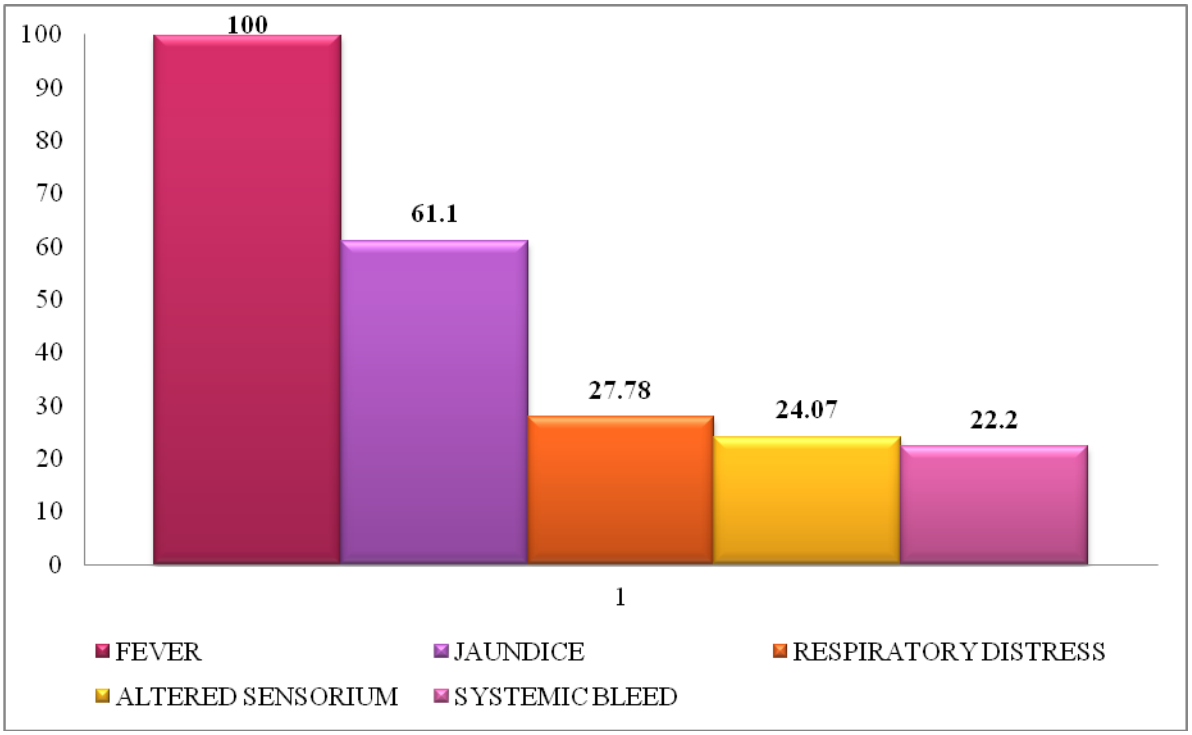


Figure 14 - Summary of clinical symptoms at admission

9.2.4 SUMMARY OF CLINICAL SIGNS

Fever was present in all patients (n = 54).Clinically evident icterus was present in 61.1%.As evident the mean values of the cohort indicate the presence of systemic inflammatory response syndrome. Hypoxia was not a common finding (n=5), though 27.78% reported symptoms of respiratory distress. The summary of findings is presented in table 4.

Summary of clinical signs at admission

Variable	**Mean**	**SD**	**Minimum**	**Maximum**
Pulse Rate(/min)	108.89	19.187	68	170
Respiratory rate(/min)	25.34	8.218	14	50
Temperature(degF)	101.460	1.9635	95.7	105
Systolic blood pressure(mm Hg)	98.91	23.565	0	160
Diastolic blood pressure(mm Hg)	61.32	61.32	0	90
Pulse oximetery (%)	95.43	4.294	80	99
GCS score	14.32	2.017	3	15

Table 4 – Summary of clinical signs at admission

9.2.5 SUMMARY OF SYSTEMIC EXAMINATION

The most common systemic examination finding was that of hepatomegaly, followed by hepatosplenomegaly, which was evident in 86.74%. 4 patients had meningeal signs positive, however patients were not subjected to CSF analysis in view of abnormal coagulation parameters.10 patients who reported respiratory distress at presentation, had bilateral crepitations but with no other features to suggest a cardiac dysfunction.2 patients had evidence of bilateral pleural effusion, on diagnostic aspiration was found to be transudative effusion. The summary of findings are summarised in Table 5.

Summary of systemic examination at admission			
Variable	**Number**	**%**	**Comments**
Cardiovascular examination **Normal** **Abnormal**	 51 3	 94.34% 5.66%	3 patients had Ischemic heart disease with moderate LV dysfunction.
Respiratory examination **Normal** **Abnormal**	 40 14	 73.5% 26.41%	The most common finding was tachypnea. 10 patients had bi-basal crepitations. 2 patients had bilateral pleural effusion. 5 patients had Hypoxia.
Abdominal examination **Normal** **Abnormal**	 8 46	 13.20% 86.74%	The most common finding was hepatomegaly, followed by hepatosplenomegaly. 3 patients had evidence of free fluid.
CNS examination **Normal** **Abnormal**	 41 13	 75.4% 24.52%	The most common finding was drowsiness. 4 patients had terminal neck stiffness.

Table 5 – Summary of systemic examination at admission

9.2.6 SUMMARY OF LABORATORY INVESTIGATIONS:

9.2.6.1 Haematological parameters:

Anaemia was noted in 51.8% of the patients with mean haemoglobin of 10.9 gm/dl. 27.7 % (n=15) required transfusion of blood products – commonly packed cells. 3 patients required multiple units of fresh frozen plasma (mean – 4) and cryoprecipitate (mean – 12).The mean leucocyte counts were within normal limits with no significant left shift.

92.4% of the patients had thrombocytopenia, which was found to be the most common haematological abnormality. The coagulation parameters were available for 11 patients, and derangement of activated partial thromboplastin time was noted. Fibrinogen was done in 2 patients who presented with overt systemic bleed requiring intensive care. Both the patients had underwent gastroscopy and colonoscopy which showed features of diffuse mucosal bleed. This was summarised in Table 6.

Haematological parameters at admission

Variable	**Mean**	**SD**	**Minimum**	**Maximum**
Haemoglobin(gm/dl	10.956	2.8121	4.1	15.4
Total leucocyte counts(cu mm)	6581.48	4047.13	1400	27200
Neutrophil count (%)	62.80	16.808	8	95
Lymphocyte count	25.24	14.02	3	79
Platelets(/cu mm)	56245.28	45770.290	6000	237000
Prothrombin Time(seconds)	13.83	2.227	11	17
INR	1.2650	0.196	1.02	1.52
Activated partial thromboplastintime(secs)	33.891	7.4923	23	48.8
Fibrinogen	196.150	133.1482	102	290.3

Table 6 – Haematological parameters at admission

9.2.6.2 Biochemical parameters:

Among the metabolic derangements, acute liver injury was the most common biochemical abnormality and was seen in 75.9 %(n= 41).Hyperbilirubinemia, predominantly unconjugated hyperbilirubinemia was seen. There was significant transaminitis noted in all patients with SGOT being more than SGPT.

Hyponatremia was seen in 64.8 %(n = 35) and hypokalemia in 20.37 %(n = 11).The most common acid base abnormality was metabolic acidosis which was seen in 72.22 %(n = 39).

Acute kidney injury was seen in 53.70 %(n = 29), with 55% having prerenal azotemia.

Blood glucose levels were available at presentation for 42 patients. Of them 6 patients had documented hypoglycaemia and 3 had hyperglycaemia.

Serum lactate dehydrogenase levels were available for 15 patients, which showed very high levels indicating the destruction caused by parasites to the red blood cells.

Procalcitonin was done in 4 patients who presented in shock, which showed a mean value of 108.92 indicating severe sepsis.

Cardiac biomarkers were available for 2 patients who were seriously ill with multi organ dysfunction requiring intensive care. The mean troponin T levels were elevated indicating a component of sepsis related myocardial dysfunction.

The findings are summarised in table 7.

Biochemical parameters at admission				
Variable	**Mean**	**SD**	**Minimum**	**Maximum**
Sodium(m mol/dl)	131.89	5.882	109	143
Potassium(m mol/dl)	4.05	0.632	3	6
Bicarbonate	19.15	5.342	4	29
Urea(mg/dl)	65.16	66.094	13	306
Creatinine(mg/dl)	1.7006	1.3605	0.57	6.54
Ph	7.3128	0.195	6.80	7.54
Procalcitonin	108.922	88.089	36.14	233.50
Total Bilirubin(mg/dl)	5.291	6.0282	0.6	27
Direct Bilirubin (mg %)	3.508	5.114	0.2	20.5
Total protein(gm/dl)	6.281	0.8714	4.8	8.1
Albumin(gm/dl)	3.130	0.6423	1.9	4.5
SGOT(U/l)	65.32	62.294	18	368
SGPT(U/l)	36.34	29.421	6	158
Alkaline phosphatase(U/l)	94.28	38.53	31	221
Glucose(mg/dl)	115.93	47.082	66	342
Lactate dehydrogenase(U/l)	1827.90	1081.16	132	4014
Creatine phosphokinase(mg/dl)	158.60	141.023	30	493
CK – MB	7.96	4.822	4.55	11.37
Troponin - T	141.06	130.58	48.72	233.40

Table 7 – Biochemical parameters at admission

9.2.7 SEVERITY OF MALARIA

74% of the cohort fulfilled the definition of severe malaria by the World Health organisation.Involvment of any organ system is considered as severe malaria.

26% had uncomplicated malaria. This summarised in Figure 15.

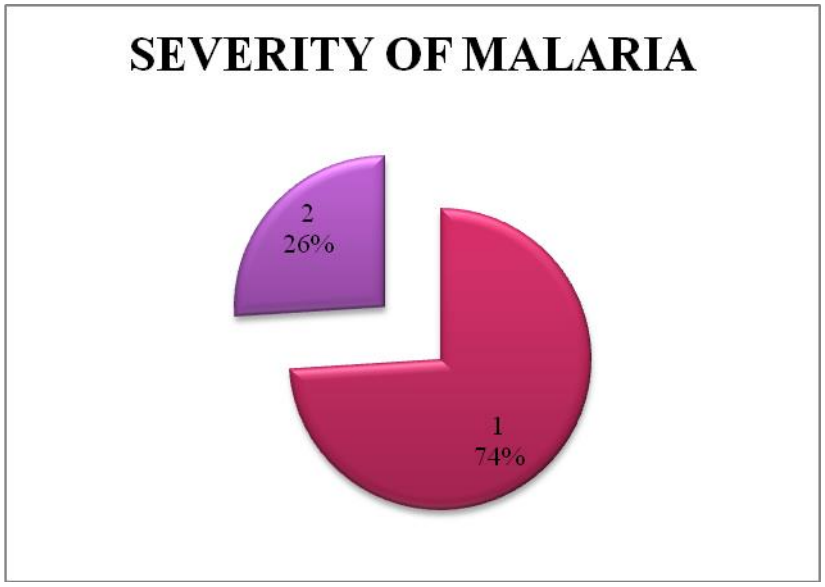


Figure 15: Severity of Malaria

1-Severe malaria, 2 – Uncomplicated malaria

9.2.7 TREATMENT REGIMEN

51.8% of the enrolled population received Intravenous artesunate with doxycycline (n=28).14.8% received oral artemether with lumefantrine (n = 8).25.92% received intravenous artesunate with doxycycline, which was subsequently changed over to oral artemether and lumefantrine (n = 14).

5 patients were given artesunate with clindamycin as rescue therapy, in view of treatment failure with artesunate and doxycycline. 11 patients received empirical broad spectrum antibiotic therapy in addition to ACT.Piperacillin – tazobactem was the commonest broad spectrum antibiotic used.

9.2.8 REQUIREMENT OF DIALYSIS

4 patients out of 54 required dialysis support in view of oliguric acute kidney injury with severe metabolic acidosis and hyperkalemia. 1 patient in addition to the above mentioned

indication required the same for hyperparasitemia with parasite index of 58%.The average duration of dialysis requirement was 3 days.

9.2.9 REQUIREMENT OF VENTILATORY SUPPORTS

40.7% of the patients in the cohort required oxygen therapy.4 patients required non invasive ventilation.4 patients required invasive mechanical ventilation, the most common indication being severe metabolic acidosis with encephalopathy. The duration of ventilatory support requirement ranged from 1 day to 7 days.1 patient had cerebral malaria complicated by hypoxic brain damage, hence underwent tracheostomy, indication being failure to wean off ventilation.

9.2.10 MORTALITY

2 patients died after recruitment in the study. 1 patient died in the emergency department due to refractory shock with severe acidosis. The other patient was admitted in intensive care unit, with severe metabolic acidosis, multi organ dysfunction syndrome in shock. He died 12 hours post admission, secondary to refractory acidosis and shock.

9.3 PRIMARY OUTCOMES

The mean parasite index at admission was 5.51 %(0.1% – 48%: SD – 11.4).The mean temperature at admission was 101.4 F.

9.3.1 PARASITE CLEARANCE TIME

Parasite clearance times were calculated for 51 patients. 3 were excluded as they did not have any detectable asexual ring forms in the peripheral smear.

25.5 %(n = 13) had delayed parasite clearance time of more than 48 hours (asexual ring stages seen in peripheral smear on day 3 of ACT). The mean estimate of the parasite clearance time was 43.049 hours (95% CI: 33.9 – 52.2).The median parasite clearance time was estimated to be 36 hours (95%CI: 27.08 – 44.91). Among the patients with delayed parasite clearance (n = 13), the mean clearance time was found to be 77.53 hours.

The cumulative parasite clearance curve - figure 16

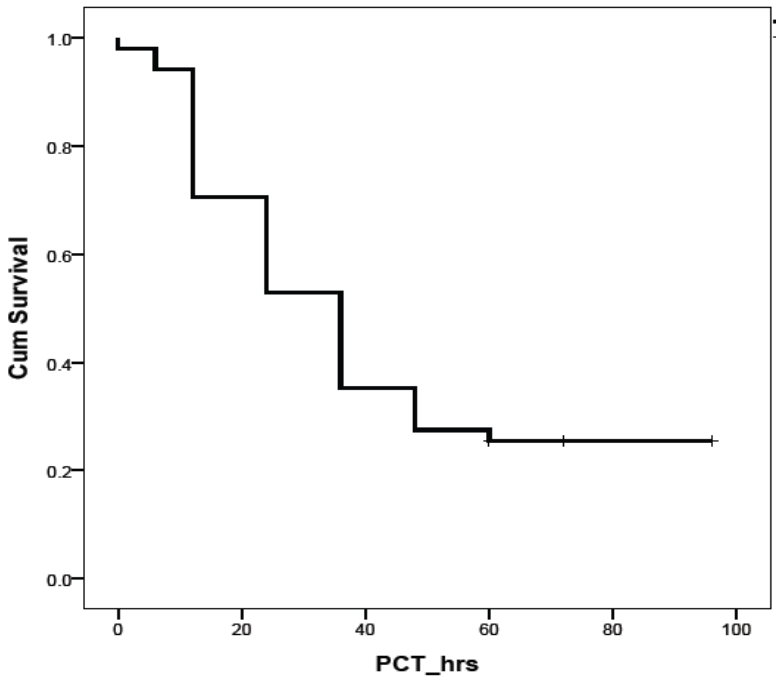


Figure 16 – Parasite clearance curve

9.3.2 FEVER CLEARANCE TIME:

Fever clearance times were calculated for 54 patients. 16.7% of patients had delayed fever clearance time (n = 9).The mean fever clearance time was 40.29 hours (95% CI: 27.78 – 52.81).The median fever clearance time was estimated to be 24 hours (95% CI: 18.69 – 29.30).Among the patients with delayed fever defervescence, the mean fever clearance time was 84.6 hours.

5 patients had relapse of fever after a period of defervescence.The mean duration of afebrile period after which they relapsed was 24 hours. The mean clearance time following relapse was 54 hours.

The cumulative fever clearance curve – figure 17

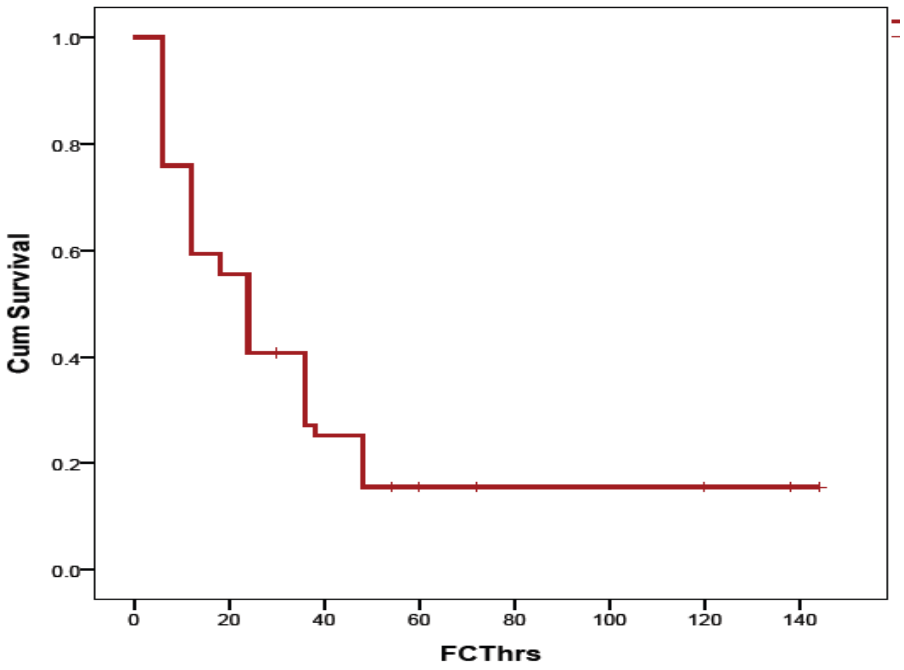


Figure 17 – Fever clearance curve

9.3.3 GAMETOCYTE CLEARANCE TIME

The mean gametocyte clearance time was estimated to be 5.4(1 – 19) days. The proportion of patients with gametocyte positivity on day 0, 3,7,14 are summarised in Figure 17.

The median gametocyte clearance time was 7 days (range: 3 -19) for patients with delayed clinical and parasite clearance times when compared to 3.5(range: 1 – 14) days in patients with adequate clinical and parasitological response. However this parameter might have been underestimated because the gametocyte clearance was assessed only up to day 7.If the patients were hospitalised more than day 7 ,they were followed up to discharge.

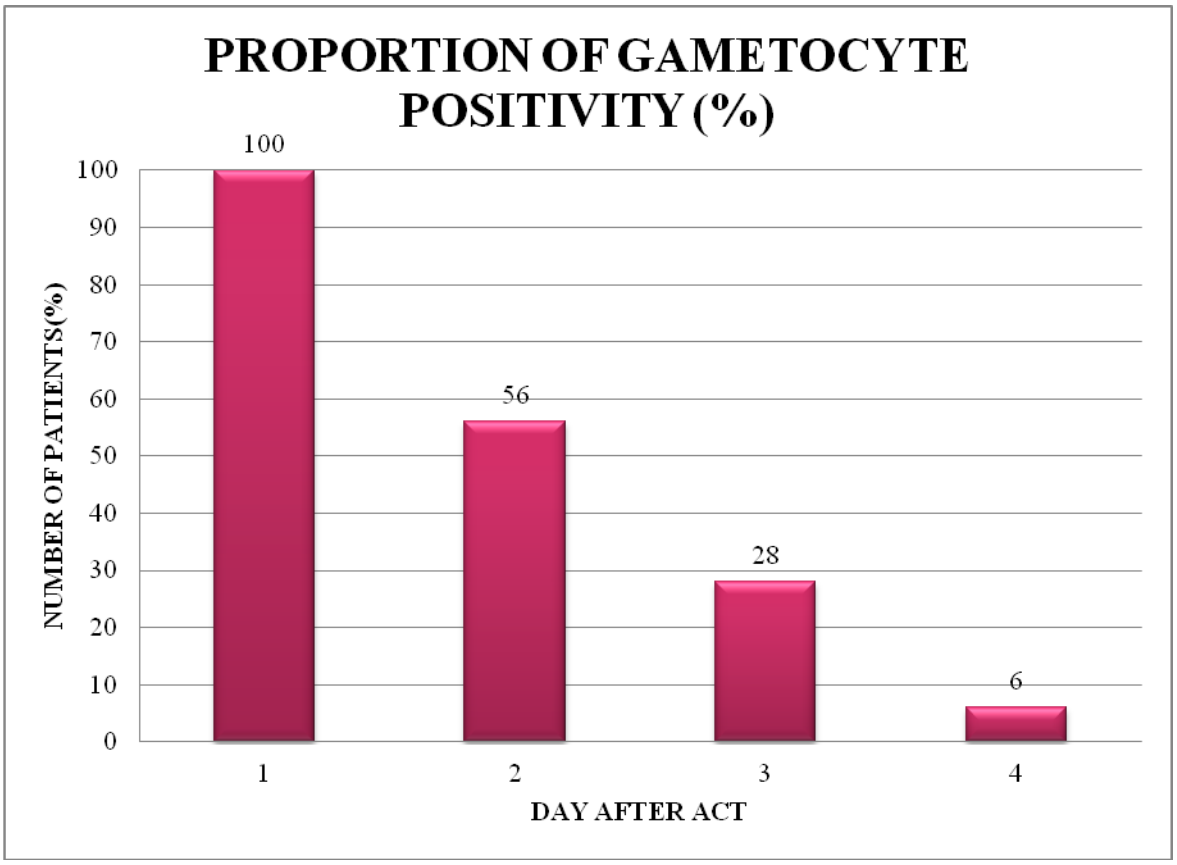


Figure 18 - Proportion of patients with detectable gametocyte vs. the days post ACT.

1 – Day 1, 2 – Day 3, 3 – Day 7, 4- Day 14

9.3.4 SUMMARY OF TREATMENT OUTCOMES

The proportion of patients with early treatment failure(47)defined as persistent parasitemia on day 3 of ACT was 25.5%.

9.3 %(n = 5) of the patients had relapse of fever post treatment for 7 days with ACT, suggestive of recrudescence. However they did not fulfil the criteria for late clinical/parasitological failure.

As per the updated definition of artemisinin resistance – August 2014(44),the proportion of patients fulfilling the criteria for partial artemisinin resistance was 25.5%.

9.4 PHARMACOKINETIC STUDIES OF INTRAVENOUS ARTESUNATE (n = 17)

The pharmacokinetic studies were done for patients hospitalised with malaria on IV artesunate.

The study was done on day 3 of initiation of artemisinin combination therapy.

The assay for measurement of drug levels was developed by the department of Clinical Pharmacology.

The number of patients recruited for the same following informed consent was 17.

2 patients were excluded from the analysis in view of different time points used for the sample collection.

The mean dose used in the patients was 2.4 mg/kg. The details of sampling and techniques are described in detail in the methodology section.

The maximum concentrations of artesunate and dihydroartemisinin, the time to peak concentrations, the levels of 4 hour drug exposure and the clearance of the drug were characterised.

The parameters were compared between patients with adequate clinical and parasitological response (n = 11) and delayed parasite clearance (n = 5), and statistical methods were used to ascertain significance.

There were no serious adverse effects noted with administration of the drug.

9.4.1 Baseline characteristics of intravenous artesunate

The mean maximum concentration attained by artesunate was 5815.03 ng/ml. It followed a linear dose relationship. The minimum level was 1878.36 ng/ml and the maximum level attained was 11989.97 ng/ml. The peak artesunate concentration was reached in 5 minutes. The rapid metabolism of the compound is evident with the rate of clearance of the drug, calculated by the formula (dose/AUC) which was 5.085 litre/kg/hour. This is summarised in table 8.

Pharmacokinetics of Intravenous artesunate

Variable	Mean	Median	Range(min – max)	SD
Maximum drug concentration(ng/ml) C_{max}	5815.033	5867.50	(1878.36 - 11989.97)	2973.36
Time to maximum concentration(mins)	5	5	-	-
AUC(0 – 4 hr exposure)	649.001	650.45	(204.67 – 1220.65)	321.66
Clearance(l/kg/hr)	5.085	-	-	2.725

Table 8 – Pk parameters of Intravenous Artesunate

9.4.2 Baseline characteristics of Dihydroartemisinin – Metabolite of Artesunate

Dihydroartemisinin is the active metabolite of artesunate.

The mean maximum concentration attained by dihydroartemisinin was 658.53 ng/ml.

It followed a linear dose relationship.

The minimum level was 180.09 ng/ml and the maximum level attained was 1274.13ng/ml.

The peak dihydroartemisinin concentration was reached in 12 minutes, probably the time taken for artesunate to completely metabolise to dihydroartemisinin.

The rate of clearance of the drug, calculated by the formula (dose/AUC) was 3.77 litre/kg/hour.

The findings are summarised in Table 9.

Pharmacokinetics of Dihydroartemisinin

VARIABLE	Mean	Median	Range (min – max)	SD
Maximum drug concentration(ng/ml) C_{max}	658.53	573.53	(180.09 – 1274.13)	345.66
Time to maximum concentration(mins)	12	12	-	-
AUC(0 – 4 hr exposure)	1053.55	884.80	(213.81 – 2654.84)	712.94
Clearance(l/kg/hr)	3.77	-	-	-

Table 9 – Pk parameters of Dihydroartemisinin

9.4.3 Pharmacokinetic parameters of artesunate and DHA among patients with adequate clinical and parasitological response

The pharmacokinetics of artesunate and dihydroartemisinin levels is tabulated for the patients with adequate clinical and parasitological response (Table 10).

The peak concentration was attained at 5 minutes for artesunate and at 12 minutes for the metabolite dihydroartemisinin.The 4 hour area under the curve was calculated which is an equivalent of the drug exposure in each patient.

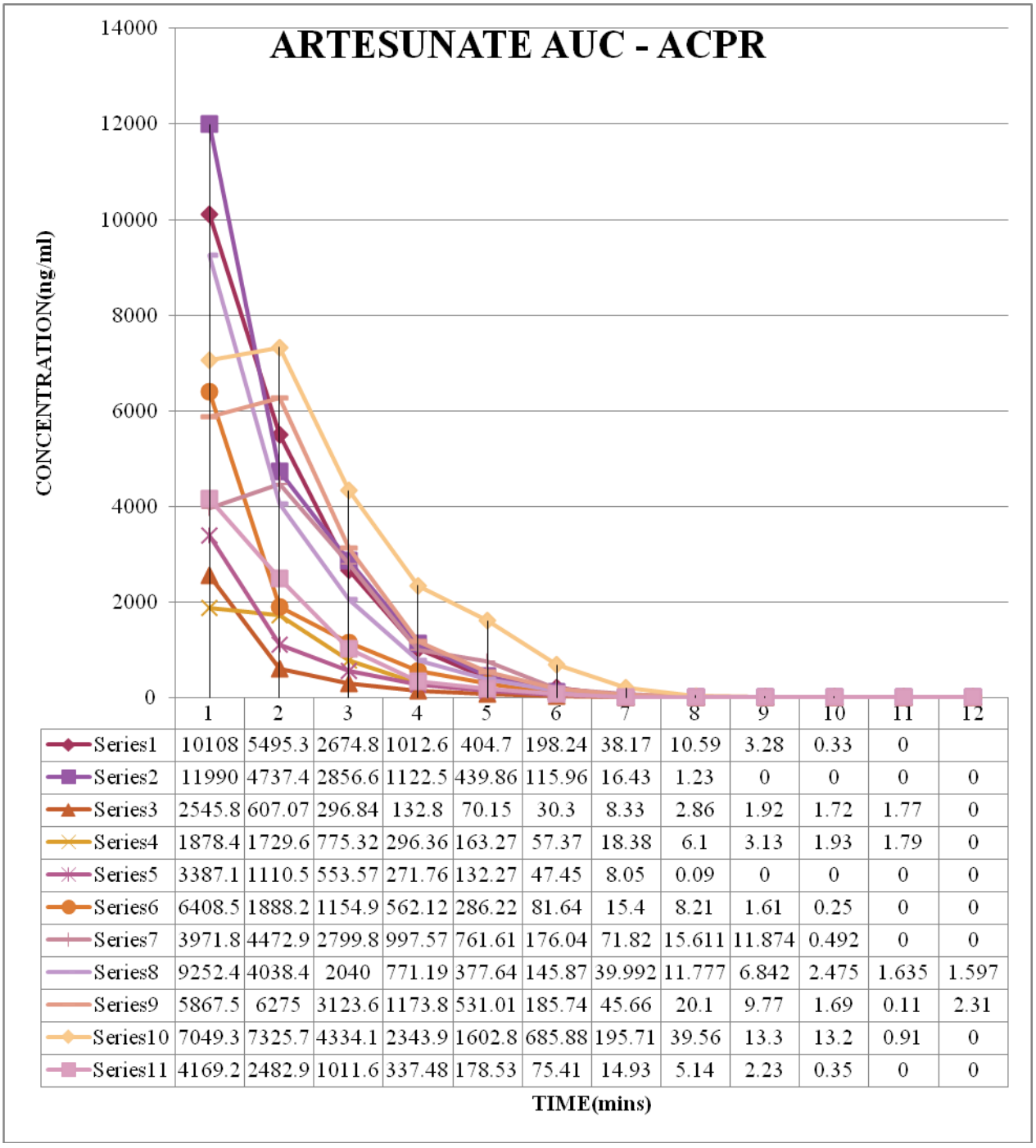
The C_{max} of artesunate was 6057.11 and that of dihydroartemisinin was 1256.66.

Pk parameters of artesunate and DHA in adequate clinical and parasitological response

Drug	**4 Hour AUC(mean)**	**4 hour AUC(median)**	**SD**	**Clearance(l/kg/hr)**	**C_{max}**
Artesunate	671.28	650.45	357.98	4.95	6057.11
DHA	1158.58	884.80	800.34	3.41	1256.66

Table 10 - PK parameters of artesunate and DHA in adequate clinical and parasitological response

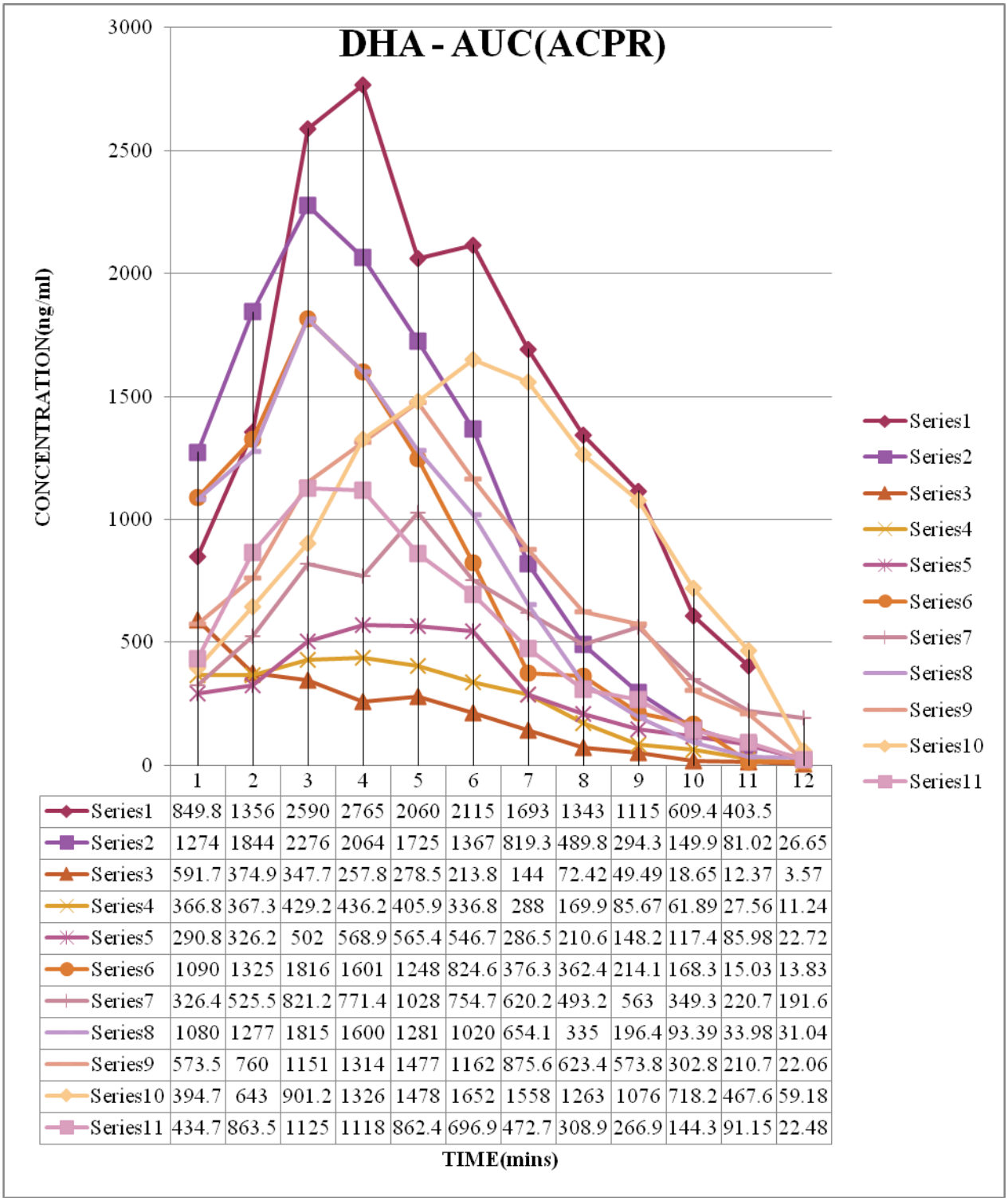
The dose distribution across time for artesunate and dihydroartemisin is summarised in Figure 18 and 19.



	1	2	3	4	5	6	7	8	9	10	11	12
Series1	10108	5495.3	2674.8	1012.6	404.7	198.24	38.17	10.59	3.28	0.33	0	
Series2	11990	4737.4	2856.6	1122.5	439.86	115.96	16.43	1.23	0	0	0	0
Series3	2545.8	607.07	296.84	132.8	70.15	30.3	8.33	2.86	1.92	1.72	1.77	0
Series4	1878.4	1729.6	775.32	296.36	163.27	57.37	18.38	6.1	3.13	1.93	1.79	0
Series5	3387.1	1110.5	553.57	271.76	132.27	47.45	8.05	0.09	0	0	0	0
Series6	6408.5	1888.2	1154.9	562.12	286.22	81.64	15.4	8.21	1.61	0.25	0	0
Series7	3971.8	4472.9	2799.8	997.57	761.61	176.04	71.82	15.611	11.874	0.492	0	0
Series8	9252.4	4038.4	2040	771.19	377.64	145.87	39.992	11.777	6.842	2.475	1.635	1.597
Series9	5867.5	6275	3123.6	1173.8	531.01	185.74	45.66	20.1	9.77	1.69	0.11	2.31
Series10	7049.3	7325.7	4334.1	2343.9	1602.8	685.88	195.71	39.56	13.3	13.2	0.91	0
Series11	4169.2	2482.9	1011.6	337.48	178.53	75.41	14.93	5.14	2.23	0.35	0	0

Figure 19 – Area under the concentration curve for artesunate in patients with adequate clinical and parasitological response.

[X axis: 1 – 5 minutes,2 – 7 minutes,3 – 9 minutes,4 – 12 minutes,5 – 15 minutes,6 – 20 minutes,7 – 30 minutes,8 – 45 minutes,9 – 60 minutes,10 – 90 minutes,11 – 120 minutes,12 – 240 minutes]



	1	2	3	4	5	6	7	8	9	10	11	12
Series1	849.8	1356	2590	2765	2060	2115	1693	1343	1115	609.4	403.5	
Series2	1274	1844	2276	2064	1725	1367	819.3	489.8	294.3	149.9	81.02	26.65
Series3	591.7	374.9	347.7	257.8	278.5	213.8	144	72.42	49.49	18.65	12.37	3.57
Series4	366.8	367.3	429.2	436.2	405.9	336.8	288	169.9	85.67	61.89	27.56	11.24
Series5	290.8	326.2	502	568.9	565.4	546.7	286.5	210.6	148.2	117.4	85.98	22.72
Series6	1090	1325	1816	1601	1248	824.6	376.3	362.4	214.1	168.3	15.03	13.83
Series7	326.4	525.5	821.2	771.4	1028	754.7	620.2	493.2	563	349.3	220.7	191.6
Series8	1080	1277	1815	1600	1281	1020	654.1	335	196.4	93.39	33.98	31.04
Series9	573.5	760	1151	1314	1477	1162	875.6	623.4	573.8	302.8	210.7	22.06
Series10	394.7	643	901.2	1326	1478	1652	1558	1263	1076	718.2	467.6	59.18
Series11	434.7	863.5	1125	1118	862.4	696.9	472.7	308.9	266.9	144.3	91.15	22.48

TIME(mins)

Figure 20 – Area under the concentration curve for dihydroartemisinin for patients with adequate clinical and parasitological response

[X axis: 1 – 5 minutes,2 – 7 minutes,3 – 9 minutes,4 – 12 minutes,5 – 15 minutes,6 – 20 minutes,7 – 30 minutes,8 – 45 minutes,9 – 60 minutes,10 – 90 minutes,11 – 120 minutes,12 – 240 minutes]

9.4.4 Pharmacokinetic parameters of artesunate and DHA among patients with delayed parasite clearance times (n=5)

The pharmacokinetic of artesunate and dihydroartemisinin levels are tabulated for the patients with delayed parasite clearance times (Table 11).

The peak concentration was attained at 5 minutes for artesunate and at 12 minutes for the metabolite dihydroartemisinin.

The 4 hour area under the curve was calculated which was the equivalent of the drug exposure in each patient.

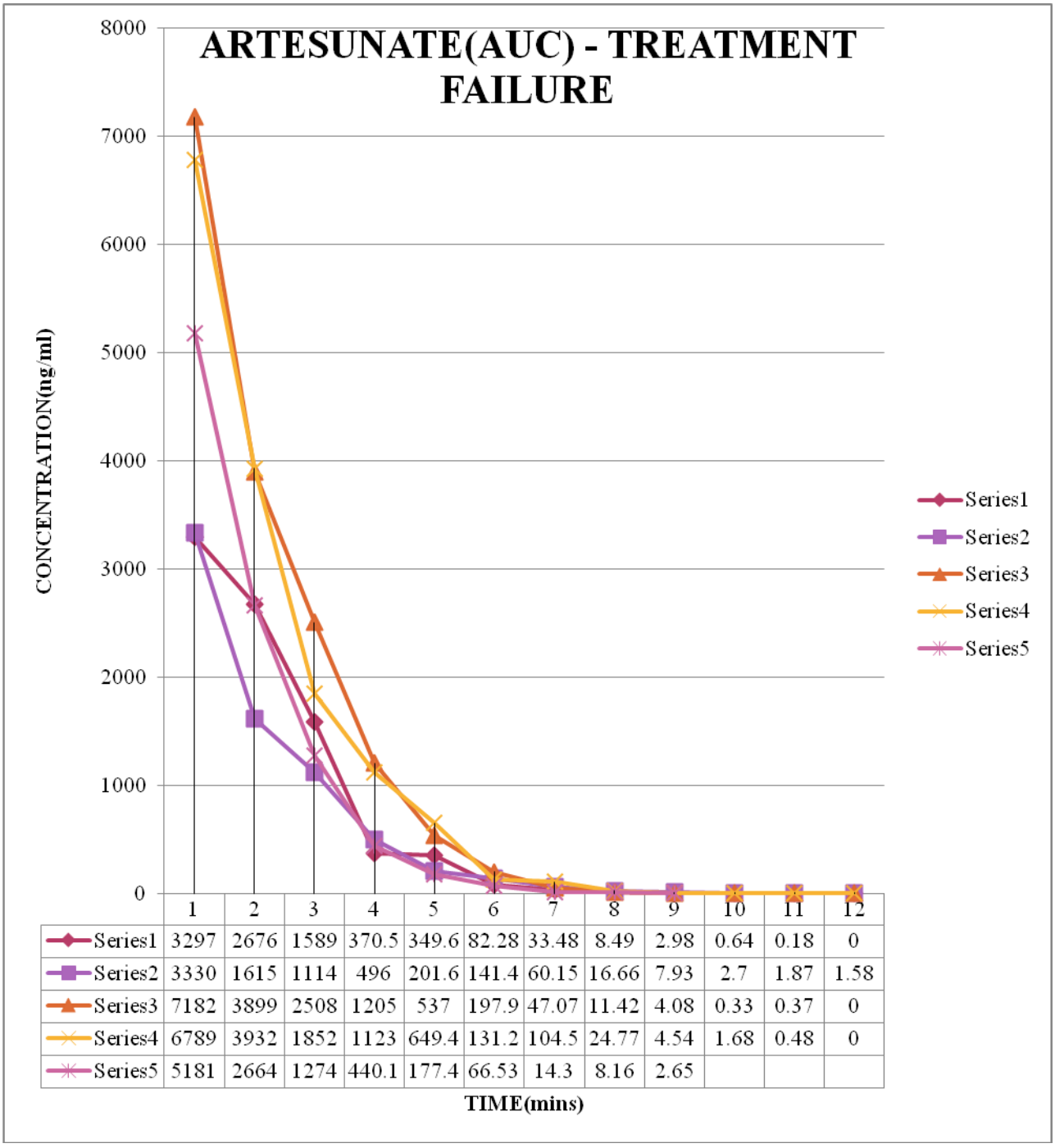
The levels of dihydroartemisinin (4 hour exposure and maximum concentration) appear to be less in patients with delayed parasite clearance times when compared to the patients with adequate clinical and parasitological response.

Pk parameters of artesunate and dihydroartemisinin among patients with treatment failure

Drug	**4 hour AUC(Mean)**	**4 hr AUC(Median)**	**SD**	**Clearance(l/kg/hr)**	**C_{max}**
Artesunate	569.77	587.17	195.60	5.22	5155.59
DHA	733.63	798.48	262.06	4.13	989.98

Table 11 - Pk parameters of artesunate and dihydroartemisinin among patients with treatment failure

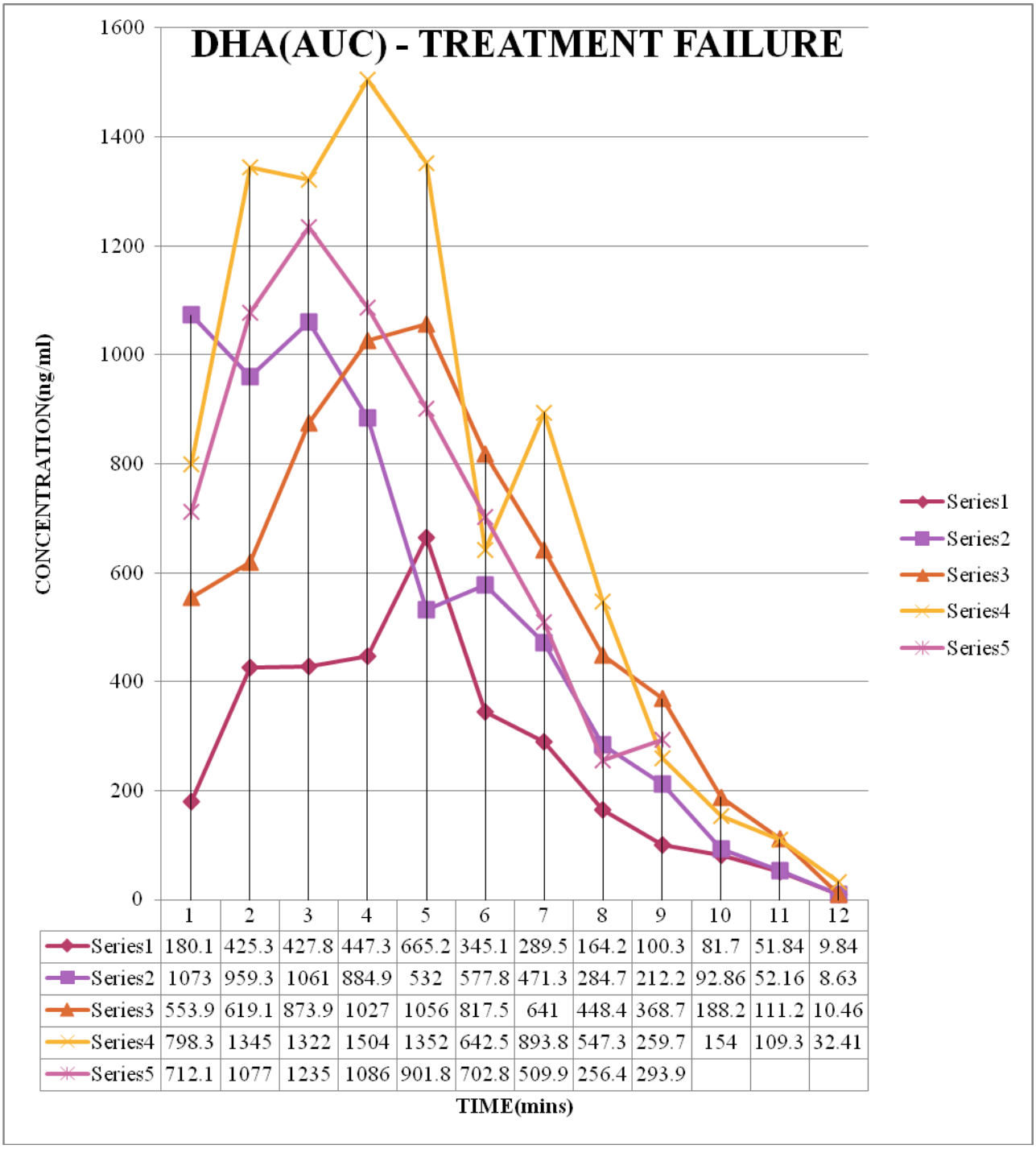
The dose distribution across time for artesunate and dihydroartemisinin is summarised in Figure 20 and 21



	1	2	3	4	5	6	7	8	9	10	11	12
Series1	3297	2676	1589	370.5	349.6	82.28	33.48	8.49	2.98	0.64	0.18	0
Series2	3330	1615	1114	496	201.6	141.4	60.15	16.66	7.93	2.7	1.87	1.58
Series3	7182	3899	2508	1205	537	197.9	47.07	11.42	4.08	0.33	0.37	0
Series4	6789	3932	1852	1123	649.4	131.2	104.5	24.77	4.54	1.68	0.48	0
Series5	5181	2664	1274	440.1	177.4	66.53	14.3	8.16	2.65			

Figure 21 – Area under the concentration for artesunate in patients with treatment failure

[X axis: 1 – trough levels,2 – 5 minutes,3 – 7 minutes,4 – 9 minutes,5 – 12 minutes,6 – 15 minutes,7 – 20 minutes,8 – 30 minutes,9 – 45 minutes,10 – 60 minutes,11 – 90 minutes,12 – 120 minutes,13 – 240 minutes]



	1	2	3	4	5	6	7	8	9	10	11	12
Series1	180.1	425.3	427.8	447.3	665.2	345.1	289.5	164.2	100.3	81.7	51.84	9.84
Series2	1073	959.3	1061	884.9	532	577.8	471.3	284.7	212.2	92.86	52.16	8.63
Series3	553.9	619.1	873.9	1027	1056	817.5	641	448.4	368.7	188.2	111.2	10.46
Series4	798.3	1345	1322	1504	1352	642.5	893.8	547.3	259.7	154	109.3	32.41
Series5	712.1	1077	1235	1086	901.8	702.8	509.9	256.4	293.9			

Figure 22 – Area under the concentration curve for dihydroartemisinin in patients with treatment failure

[X axis: 1 – 5 minutes,2– 7 minutes,3 – 9 minutes,4– 12 minutes,5 – 15 minutes,6– 20 minutes,7 – 30 minutes,8– 45 minutes,9 – 60 minutes,10 – 90 minutes,11 – 120 minutes,12 – 240 minutes]

9.4.5 INTER PERSON VARIABILITY OF DRUG CONCENTRATIONS

There was significant inter person variability noted among the subjects, for the concentrations of artesunate and dihydroartemisinin.

This was calculated using the formula :(Standard deviation/Mean)*100.

This summarised in Table 12.

INTER PERSON VARIABILITY OF PHARMACOKINETIC PARAMETERS

VARIABLE	ARTESUNATE VARIABILITY	DIHYDROARTEMISININ VARIABILITY
RESPONDERS	54.9%	67.7%
TREATMENT FAILURES	36.7%	37.8%

Table 12 – INTER PERSON VARIABILITY OF PHARMACOKINETIC PARAMETERS

9.5 SUMMARY OF UNIVARIATE ANALYSIS – COMPARISON WITH ADEQUATE PARASITOLOGICAL RESPONSE AND DELAYED PARASITE CLEARANCE TIMES

The baseline characteristics including the demographic variables, haematological, biochemical parameters were compared to identify factors which are associated with delayed parasite clearance times. This was done using the T test for equality of means and Levene's test for equality of variances. This is tabulated as follows :(APR – adequate parasitological response, DPR – delayed parasitological response)

9.5.1 DEMOGRAPHIC VARIABLES

Demographic variables which were compared between patients with adequate parasitological response and delayed clearance include occupation, history of travel to endemic areas and

geographic location. In view of the male preponderance, gender was not included in the univariate analysis.The distribution of patients with delayed clinical as well as parasitological clearance across different groups of occupation was similar. The difference was tested with Pearson's Chi square test and was not found to be statistically significant.The history of travel to malaria endemic areas as well as state of residence(Tamilnadu,Andhra Pradesh) were also correlated with Pearson's Chi square test and was found to be similar between the groups.

9.5.2 CLINICAL VARIABLES

The vital signs, especially the tachycardia and hypotension appear to be severe in the group with delayed parasitological clearance. However the difference in the clinical variables was correlated with t test, and was not found to be statistically significant between the two groups. The findings are summarised in Table 12.

CLINICAL VARIABLES ASSOCIATED WITH DELAYED PARASITOLOGICAL RESPONSE

Summary of univariate analysis

VARIABLE	**MEAN(APR) [+_sd]**	**MEAN(DPR) [+_sd]**	**95% CI (diff)**	**p VALUE**
Age(years)	37.88(15.462)	36.69(9.268)	-7.9 – 10.3	0.739
Pulse rate(bts/min)	107.88(17.762)	112(23.580)	16.4 – 8.2	0.570
Respiratory rate(Breaths/min)	24.98(7.898)	26.46(9.386)	-8.9 – 3.1	0.613
Systolic BP(mm Hg)	101.05(20.566)	92.31(31.132)	-13.4 – 21.4	0.358
Diastolic BP(mm Hg)	62.25(18.603)	58.46(18.187)	12.0 – 15.2	0.524
Oxygen saturation	95.65(4.035)	94.77(5.134)	-2.5 – 3.8	0.580
Temperature(F)	101.4(2.107)	101.6(1.49)	-2.3 – 0.5	0.647

APR – Adequate parasitological response, DPR – Delayed parasitological response,DIFF – Difference, CI – Confidence interval

Table 13 – Univariate analysis - CLINICAL VARIABLES ASSOCIATED WITH DELAYED PARASITOLOGICAL RESPONSE

9.5.3 HAEMATOLOGICAL PARAMETERS

The severity of anaemia and thrombocytopenia was greater in patients with delayed parasite clearance times; however the mean difference was not statistically significant.

The type of infection – *falciparum* and mixed infection (*falciparum* and *vivax*) were correlated with delayed clearance, using the Fisher's exact test and did not show any association with delayed clearance. This is summarised in Table 13.

HAEMATOLOGICAL PARAMETERS ASSOCIATED WITH DELAYED PARASITOLOGICAL RESPONSE

Summary of univariate analysis

VARIABLE	**MEAN (APR)[+_sd]**	**MEAN (DPR)[+_sd]**	**MEAN DIFF**	**95% CI**	**P VALUE**
Haemoglobin (gm/dl)	11.02(2.91)	10.73(2.56)	0.29	-1.51 – 2.10	0.729
Total leukocyte counts(cu mm)	6314.63 (4370.04)	7423.08 (2770.12)	-1108.43	-3699.8 – 1482.9	0.289
Platelets(cu mm)	61250 (47835.58)	40846 (36066.71)	20403.84	-8657.2 – 49464.9	0.115
Prothrombin time(seconds)	13.6(2.34)	14.43(2.18)	-0.833	-4.37 – 2.70	0.611
INR	1.23(0.19)	1.39(0.17)	-0.162	-0.51 – 0.194	0.391
Activated partial thromboplastin time(seconds)	33.4(8.58)	35.1(4.38)	-1.75	-13.7 – 10.2	0.670

APR – Adequate parasitological response, DPR – Delayed parasitological response,DIFF – Difference, CI – Confidence interval

Table 14 – Univariate analysis - HAEMATOLOGICAL PARAMETERS ASSOCIATED WITH DELAYED PARASITOLOGICAL RESPONSE

9.5.4 BIOCHEMICAL PARAMETERS

On comparing the biochemical parameters, severe acute kidney injury and severe acute liver injury was noted in patients with delayed parasite clearance times as compared to the patients with adequate parasitological response. The bicarbonate levels were significantly lower in patients with delayed response, which was found to be statistically significant. This is summarised in Table 14

BIOCHEMICAL PARAMETERS ASSOCIATED WITH DELAYED PARASITOLOGICAL RESPONSE
Summary of univariate analysis

VARIABLE	**MEAN APR (+_sd)**	**MEAN DPR (+_sd)**	**MEAN DIFF.**	**95% CI**	**P VALUE**
Glucose(mg/dl)	115.42(32.9)	117.36(76.3)	-1.9	-35.7 – 31.8	0.936
Urea (mg%)	51.13(52.2)	106.15(85.6)	-55	-95.1 - -14.9	0.045
Creatinine(mg/dl)	1.46(1.1)	2.43(1.8)	-0.96	-1.8 - -0.13	0.087
Sodium(mmol/dl)	131.08(6.014)	132.15(5.6)	-0.354	-4.16 – 3.45	0.849
Potassium(mmol/dl)	4.01(0.62)	4.20(0.65)	-0.195	-0.6 – 0.21	0.362
Bicarbonate(mmol/dl)	20.08(4.81)	16.16(6.06)	3.919	0.52 – 7.31	0.058
Total Bilirubin (mg %)	4.16(5.31)	8.75(6.96)	-4.58	-8.26 - -0.9	0.016
Direct Bilirubin (mg %)	2.16(4.53)	6.24(5.97)	-3.68	-6.77 - -0.47	0.025
Total Protein (mg %)	6.39(0.81)	5.93(0.98)	0.454	- 0.09 – 1	0.103
Serum albumin (mg %)	3.21(0.63)	2.87(0.61)	0.335	-0.06 – 0.74	0.102
Aspartate transferase(U/l)	47.43(36.3)	120.38(90.4)	-72.96	-107.6 – 38.2	0.00
Alanine transferase(U/l)	30.63(22.9)	53.92(32.87)	-23.29	-41.77 - -5.41	0.012
Alkaline phosphatase(U/l)	96.53(41.82)	87.38(29.2)	9.14	-15.66 – 33.94	0.463
Lactate dehydrogenase(U/ml)	2000.07(1111.4)	1354.43(964.2)	645.64	-715.69 – 2006.94	0.324

APR – Adequate parasitological response, DPR – Delayed parasitological response,DIFF – Difference, CI – Confidence interval

Table 15 – Univariate analysis - BIOCHEMICAL PARAMETERS ASSOCIATED WITH DELAYED PARASITOLOGICAL RESPONSE

9.6 SUMMARY OF UNIVARIATE ANALYSIS – COMPARISON WITH ADEQUATE CLINICAL RESPONSE AND DELAYED FEVER CLEARANCE TIMES

Delayed fever clearance time was defined fever defervescence more than 48 hours. The demographic variables, haematological, biochemical parameters were compared to identify factors which have a correlation with delayed fever clearance times. This was done using the T test for equality of means and Levene's test for equality of variances. This is tabulated as follows :(ACR – adequate clinical response, DPR – delayed clinical response).

9.6.1 DEMOGRAPHIC VARIABLES

Demographic variables like occupation, history of travel to endemic areas and geographic location were compared between patients with adequate clinical response and delayed clearance.

In view of the male preponderance, gender was not included in the univariate analysis.

The distribution of patients with delayed clinical as well as parasitological clearance across different groups of occupation was similar. The difference was tested with Pearson's Chi square test and was not found to be statistically significant.

The history of travel to malaria endemic areas as well as state of residence(Tamilnadu,Andhra Pradesh) were also correlated with Pearson's Chi square test and was found to be similar between the groups.

9.6.2 CLINICAL VARIABLES:

The clinical variables are comparable between the two groups. The mean temperature at admission was 101 F in those with adequate clinical response and 102.2 F in those with prolonged fever defervescence.Both groups had tachycardia with low systolic blood pressures. There was no statistical difference between the two groups.This is summarised in Table 15.

CLINICAL VARIABLES ASSOCIATED WITH DELAYED CLINICAL RESPONSE

Summary of univariate analysis

VARIABLE	**MEAN-ACR (+_sd)**	**MEAN-DCR (+_sd)**	**MEAN DIFF**	**p VALUE**
Age(years)	37.29(14.8)	39.11(10.1)	-1.8	0.728
Pulse rate(bts/min)	109.7(20.2)	104.89(13.1)	4.8	0.498
Respiratory rate(breaths/min)	24.84(8.5)	27.78(6.2)	-2.9	0.333
Systolic BP(mm Hg)	99.59(25.1)	95.56(14.2)	4.0	0.644
Diastolic BP(mm Hg)	61.59(19.7)	60(10)	1.5	0.816
Oxygen Saturation	95.55(4.6)	94.89(2.3)	0.65	0.680
Temperature(F)	101.305(2.03)	102.22(1.43)	-0.91	0.204

ACR – Adequate clinical response, DCR – Delayed clinical response,DIFF – Difference, CI – Confidence interval

Table 16 – Univariate analysis - CLINICAL VARIABLES ASSOCIATED WITH DELAYED CLINICAL RESPONSE

9.6.3 HAEMATOLOGICAL PARAMETERS

On univariate analysis, the number of platelets appears to be different between the groups with 61795 in patients with adequate clinical response and 29111 in patients with prolonged fever defervescence.This difference was found to be statistically significant. The other parameters were comparable and no statistically significant difference was identified between the groups. This is summarised in Table 16.

HAEMATOLOGICAL PARAMETERS ASSOCIATED WITH DELAYED CLINICAL RESPONSE
Summary of univariate analysis

VARIABLE	**MEAN-ACR (+_sd)**	**MEAN-DCR (+_sd)**	**MEAN DIFF.**	**95% CI**	**P VALUE**
Haemoglobin(gm/dl)	11.02(2.9)	10.6(1.9)	0.4	-1.6 – 2.4	0.701
Total leukocyte counts (cu mm)	6500(4269.9)	6988.8(2829.9)	-488.889	-3479.6 – 2501.8	0.744
Platelets(cu mm)	61795.45 (47182.2)	29111.11 (25565.8)	32684.3	7.447 – 65361.240	0.050
Prothrombin time(seconds)	14.07(2.4)	12.75(0.35)	1.317	-2.71 – 5.34	0.479
INR	1.27(0.2)	1.19(0)	0.08	-0.41 – 0.58	0.712
Activated partial thromboplastin time(seconds)	34.9(7.98)	29.1(1.48)	5.74	-7.467 – 19.05	0.349

ACR – Adequate clinical response, DCR – Delayed clinical response,DIFF – Difference, CI – Confidence interval

Table 17 – Univariate analysis - HAEMATOLOGICAL PARAMETERS ASSOCIATED WITH DELAYED CLINICAL RESPONSE

9.6.4 BIOCHEMICAL PARAMETERS:

Among the biochemical parameters, the level of acute kidney injury, acute hepatic injury with hyperbilirubinemia and hypoalbuminemia appeared to be different between the two groups. The severity was higher in the group with delayed defervescence.The mean difference was found to be statistically significant. This is summarised in Table 17.

BIOCHEMICAL PARAMETERS ASSOCIATED WITH DELAYED CLINICAL RESPONSE

Summary of univariate analysis

VARIABLE	**MEAN-ACR (+_sd)**	**MEAN-DCR (+_sd)**	**MEAN DIFF**	**95% CI**	**p VALUE**
Glucose(mg/dl)	112(31.9)	139.5(101.29)	-27.5	-69.06 – 14.06	0.189
Creatinine(mg/dl)	1.5(1.18)	2.9(1.83)	-1.08	-2.04 - -0.12	0.027
Urea (mg%)	56.84(58.1)	109.88(90.4)	-53.08	-102.4 - -3.67	0.036
Sodium(m mol/dl)	131.89(5.99)	131.89(5.62)	-0.003	-4.36 – 4.36	0.999
Potassium(m mol/dl)	4.03(0.604)	4.16(0.789)	-0.124	-0.591 – 0.344	0.597
Bicarbonate	19.5(5.44)	17.5(4.79)	1.94	-2.002 – 5.886	0.327
Total Bilirubin (mg %)	4.57(5.75)	8.81(6.45)	-4.23	-9.365 – 0.866	0.095
Direct Bilirubin (mg %)	2.84(4.76)	6.76(5.78)	-3.92	-8.493 – 0.641	0.085
Total protein (mg %)	6.38(0.885)	5.76(0.602)	0.61	0.108 – 1.13	0.020
Serum Albumin	3.20(0.665)	2.76(0.350)	0.43	0.118 – 0.757	0.009
Alkaline phosphatase(U/l)	91.89(39.47)	106(32.99)	0.279	-41.09 – 12.8	0.279
Lactate dehydrogenase(U/l)	1860.76(1212.42)	1778.62(957.17)	82.13	-1137.42 – 1301.70	0.886

ACR – Adequate clinical response, DCR – Delayed clinical response,DIFF – Difference, CI – Confidence interval

Table 18 – Univariate analysis - BIOCHEMICAL PARAMETERS ASSOCIATED WITH DELAYED CLINICAL RESPONSE

9.7 PARASITE INDEX AT ADMISSION

The parasite index at admission was compared between two groups of adequate clinical and parasitological response (n = 35) and delayed parasite and fever clearance (n = 18)(Figure 22).The difference was compared using the Mann Whitney U test. The difference was found to be statistically significant (p value – 0.027)

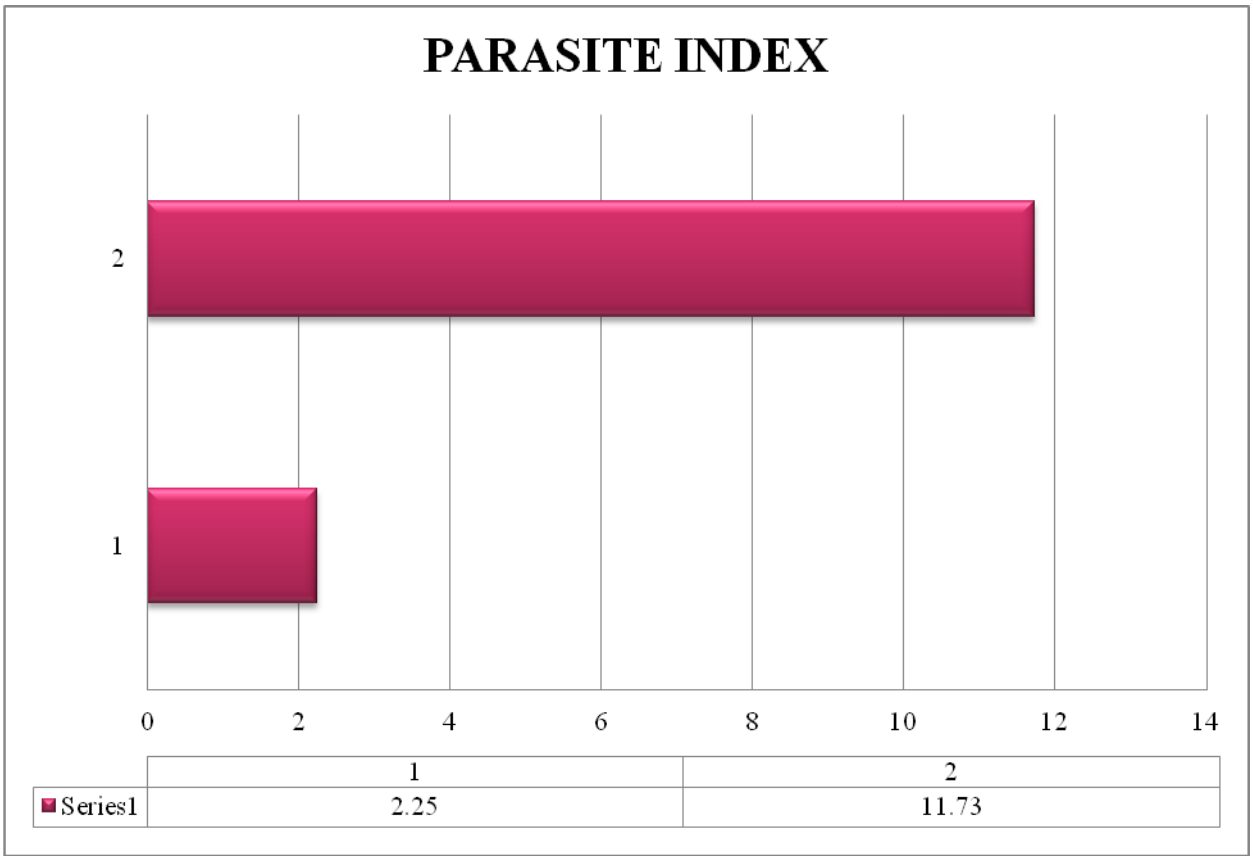


Figure 23 – Parasite Index at admission 1 - Adequate Clinical and Parasitological Response, 2 – Delayed Clinical and Parasitological Response

9.8 UNIVARIATE ANALYSIS OF THE DRUG LEVELS (4 HOUR AUC) EXPOSURE – COMPARISON BETWEEN PATIENTS WITH DELAYED PARASITE CLEARANCE TIME AND ADEQUATE PARASITOLOGICAL RESPONSE

(APR: Adequate parasitological response, DPR: Delayed parasitological response)

The mean drug levels measured as the 4 hour area under the concentration curve obtained from the pharmacokinetic studies of artemisinin and metabolite dihydroartemisinin between patients with adequate parasite response(n = 4) and delayed parasite clearance(n = 4)was compared using the Mann – Whitney U test.

The results are summarised in Table 18.

DRUG CONCENTRATIONS AND TREATMENT FAILURE

Summary of univariate analysis

VARIABLE(number)	MEAN (APR)	STD DEVIATION (APR)	MEAN (DPR)	STD DEVIATION (DPR)	P - VALUE
ARTESUNATE (11)	671.28	357.98	587.73	221.05	0.661
DIHYDRO – ARTEMISININ(4)	1158.58	800.34	764.70	291.77	0.571

APR – Adequate parasitological response, DPR – Delayed parasitological response,DIFF – Difference, CI – Confidence interval

Table 19 – Univariate analysis - DRUG CONCENTRATIONS AND TREATMENT FAILURE

The drug levels though appear to have lower levels in patients with delayed parasite clearance, this difference was not statistically significant. However the numbers in each arm would be a potential limitation to make any standard conclusion.

9.9 SUMMARY OF LOGISTIC REGRESSION ANALYSIS – COMPARISON BETWEEN PATIENTS WITH ADEQUATE PARASITOLOGICAL RESPONSE AND DELAYED PARASITE CLEARANCE TIMES

The demographic variables, haematological parameters and biochemical parameters were categorically classified and a logistic regression analysis was done to identify those which might have an association or a trend towards causing a delay in parasite clearance times.

The variables which were found to be statistically significant in univariate analysis were: age, creatinine, platelet counts, and parasite index at admission, total bilirubin and direct bilirubin levels. Age > 30 years was found to have an odds ratio of 5.717(95% CI – 0.845 – 38.9) which was not found to be statistically significant (p value – 0.075).This is summarised in Table 19.

FACTORS ASSOCIATED WITH TREATMENT FAILURE(DELAYED PARASITOLOGICAL RESPONSE)

Summary of multivariate logistic regression analysis

VARIABLE	VALUE	ODDS RATIO	95% CI	p VALUE
Age(years)	30	5.717	(0.84 – 38.9)	0.075
Platelet counts(cu mm)	50,000	2.217	(0.42 – 11.5)	0.343
Creatinine(mg/dl)	1.2	1.436	(0.28 – 7.33)	0.664
Parasite index at admission (%)	5	1.038	(0.95 – 1.12)	0.373
Total Bilirubin (mg %)	2	1.480	(0.81 – 2.63)	0.200
Direct Bilirubin (mg%)	0.5	0.740	(0.38 – 1.42)	0.368

Table 20 – Multivariate logistic regression analysis - FACTORS ASSOCIATED WITH TREATMENT FAILURE (DELAYED PARASITOLOGICAL RESPONSE)

The other parameters showed a positive odds ratio of association with delayed parasitological clearance; however it was not found to be statistically significant.

The state/geographical area was categorised into two groups – TamilNadu and Andhra Pradesh. There was trend towards patients from Andhra Pradesh to have delayed parasite clearance times. The precise location is marked in the map (Figure 23, 24)

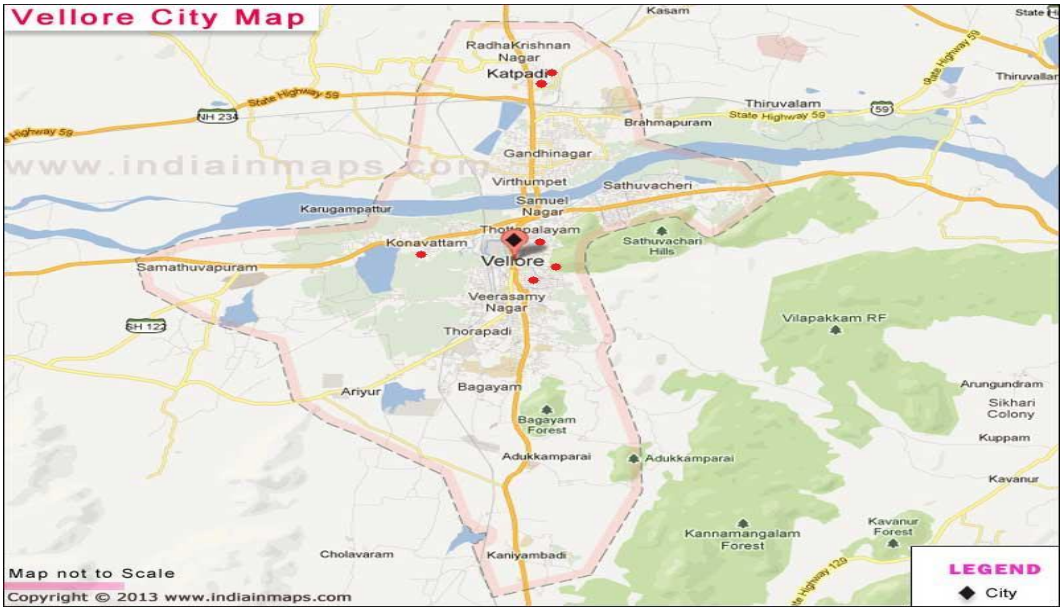


Figure 24: Vellore map – Location of treatment resistant cases

- ***Location of patients with delayed parasite clearance times***

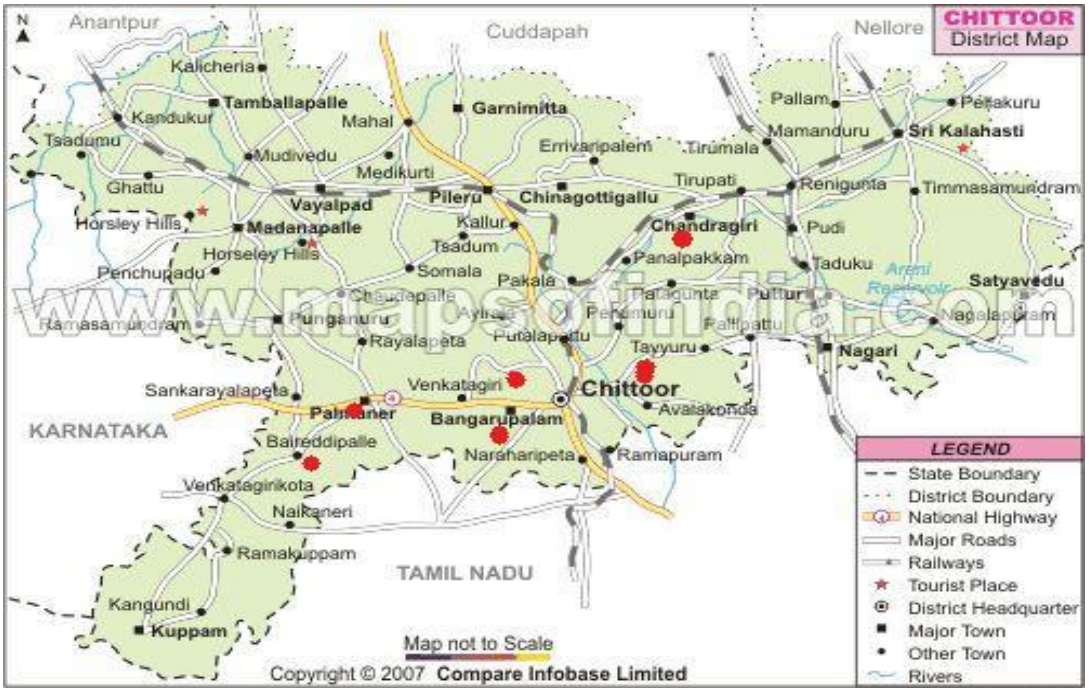


Figure 25 – Chittoor map – Location of treatment resistant cases

9.10 SUMMARY OF LOGISTIC REGRESSION ANALYSIS – COMPARISON BETWEEN PATIENTS WITH ADEQUATE CLINICAL RESPONSE AND DELAYED FEVER CLEARANCE TIMES

The demographic variables, haematological parameters and biochemical parameters were categorically classified and a logistic regression analysis was done to identify those which might have an association or a trend towards causing a delay in parasite clearance times. The variables which were found to be statistically significant in univariate analysis were: age, creatinine, platelet counts, and parasite index at admission, total bilirubin and direct bilirubin levels. Multivariate analysis showed a trend towards significance for thrombocytopenia and parasite index at admission to contribute to delay in parasite clearance times; however the difference was not statistically significant. This is summarised in Table 20.

FACTORS ASSOCIATED WITH TREATMENT FAILURE(DELAYED CLINICAL RESPONSE)

Summary of Multi variate logistic regression analysis

VARIABLE	VALUE	ODDS RATIO	95% CI	p VALUE
Age(years)	30	2.240	(0.31 – 16.1)	0.423
Platelet counts(cu mm)	50,000	3.416	(0.47 – 24.4)	0.222
Creatinine (mg %)	1.2	2.710	(0.375 – 19.6)	2.710
Parasite index at admission (%)	5	1.063	(0.98 – 1.14)	0.125
Total Bilirubin (mg%)	1.5	0.766	(0.39 – 1.48)	0.428
Direct Bilirubin (mg%)	0.5	1.449	(0.69 – 1.00)	0.318

Table 21 – Multivariate logistic regression analysis – FACTORS ASSOCIATED WITH TREATMENT FAILURE

9.12 SUMMARY OF LOGISTIC REGRESSION ANALYSIS – COMPARISON OF HOST FACTORS WITH DRUG CONCENTRATIONS

The mean exposure concentration of artesunate and dihydroartemisinin (active metabolite) measured by the area under concentration curve (0 – 240 mins) were compared with parasite clearance, parasite index at admission and the presence of acute kidney injury (elevated creatinine), to determine whether the drug concentrations of artesunate and the metabolite are different between:

1) Responders and patients with delayed parasite clearance

2) Patients with higher parasite index (PI>2%)

3) Patients with renal failure (creatinine > 1.2 gm %)

This was analysed using the multi variate logistic regression model if the drug concentrations vary in each of the above mentioned group.

9.12.1 ARTESUNATE MEAN AUC $^{(0-240)}$ CONCENTRATIONS

The artesunate concentrations in patients with treatment failure were lower than those with adequate response. The mean difference showed a trend towards significance.

The artesunate drug concentration was different between those with hyper parasitemia (PI>2%) and lower levels of parasitemia.This was found to be statistically significant.

The drug levels were found to be higher in patients with renal failure, but the difference was not significant between the groups. The results for artesunate are summarised in table 21.

FACTORS CONTRIBUTING TO DIFFERENCE IN ARTESUNATE LEVELS

VARIABLE	**MEAN DIFFERENCE(ng/ml)**	**95% CI**	**p VALUE**
Delayed parasite clearance	-285.42	-683.30 – 112.45	0.160
Parasite Index at admission	24.2	6.83 – 41.66	0.006
Creatinine	-5.083	-138.44 – 128.27	0.940

Table 22 –Multi variate logistic regression - FACTORS CONTRIBUTING TO DIFFERENCE IN ARTESUNATE LEVELS

9.12.2 DIHYDROARTEMISININ (ACTIVE METABOLITE) MEAN AUC $^{(0-240\ mins)}$ CONCENTRATIONS

The dihydro artemisinin (active metabolite of artesunate) concentration in patients with treatment failure was also found to be lower than those with adequate response. The mean difference showed a trend towards significance.

The dihydroartemisinin drug concentration was different between those with hyper parasitemia (PI>2%) and lower levels of parasitemia.This was found to be statistically significant.

The drug levels were found to be higher in patients with renal failure, but the difference was not significant between the groups. The results for dihydroartemisinin are summarised in Table 22.

FACTORS CAUSING DIFFERENCE IN DIHYDROARTEMISININ LEVELS

VARIABLE	**MEAN DIFFERENCE(ng/ml)**	**95% CI**	**p VALUE**
Delayed parasite clearance	-197.84	-450.76 – 55.07	0.125
Parasite Index at admission	14.63	2.712 – 26.563	0.016
Creatinine	7.739	-63.801 – 79.28	0.832

Table 23 - Multi variate logistic regression - FACTORS CONTRIBUTING TO DIFFERENCE IN DIHYDROARTEMISIN LEVELS

9.13 FACTORS ASSOCIATED WITH TREATMENT FAILURE - SUMMARY OF BOOTSTRAP ANALYSIS

In view of the small sample size, a boot strap analysis was done to extrapolate those variables which were found significant or showed a trend towards significance to a larger population containing the same characteristics of the cohort.

The variables with p values <0.2 was taken into consideration. This included age, platelet counts, parasite index at admission, creatinine, total bilirubin and direct bilirubin.

Age was found to be the only variable which was found to have difference in comparing patients with adequate parasitological response and treatment failure which was statistically significant [OR 1.7,p value – 0.012)

The other variables were not found to have significant association with treatment failure.

10. DISCUSSION

The advent of artemisinin based combination therapy has revolutionised the management of severe malaria, with significant mortality benefits when compared to quinine.(16, 17).The dynamic nature in which they rapidly clear the asexual forms of parasites and also block the pathogenesis of micro vascular sequestration, causing further host damage, make them the first line agents against this tropical disease. This further promoted the use of monotherapy, as well as marketing and distribution of 'below the quality' drugs containing minimal amounts of artemisinin compounds leading to development of resistant strains(1,7,43,54).

The present study describes a cohort of patients with malaria either caused only by *falciparum* species or as a mixed infection with *vivax* species presenting to a tertiary care referral hospital in Vellore, Tamil Nadu.

The sample size was calculated assuming a 25% prevalence of treatment failure to artemisinin combination therapy, which was 75.However between the recruitment periods 2012 – 2014, only 57 patients of slide confirmed *falciparum* malaria presented to our institution. This can be explained by the remarkably declining trend in the malaria cases in India as well in the state of Tamil Nadu,secondary to intensive preventive strategies including vector control measures(9,11).

There might be a potential referral bias, as patient recruitment was institution based, which mainly caters to referred cases from different places in Tamil Nadu, and the neighbouring state Andhra Pradesh. The catchment areas are Vellore and surrounding areas and Chittoor in Andhra.

10.1 TREATMENT FAILURE WITH ARTEMISININ COMBINATION THERAPY

The first correspondence by Noedl et al in 2008 of delayed parasite clearance times in western Cambodia(6),was described as ‘not so widespread epidemiological occurrence but might be a concern’. However in less than a year’s time, it promoted series of community based surveillance studies by Dondorp et al(5,54), identifying one of the biggest threats in management of malaria ‘ Artemisinin resistance’.

The proportion of patients with early treatment failure to artemisinin therapy in our cohort defined as presence of parasitemia(asexual ring stages on day 3 of ACT)(47),and as per the present updated definition, the proportion of patients fulfilling the criteria for partial artemisinin resistance(44) was 25.5%.

The median parasite clearance time for the cohort was estimated to be 36 hours and median fever clearance time was 24 hours, which indicates the high overall efficacy of artemisinin for rapid clearance of the parasites by 48 hours.

Among the patients with delayed parasite clearance, the mean clearance time was found to be 77.53 hours. All the patients cleared parasites by 90 hours, and the maximum noted fever clearance time was 144 hours.

Though the overall performance of artesunate in the form of rapidity of fever and parasite clearance is impressive, the proportion of patients with persistence of parasitemia is an alarming sign which requires further investigation to confirm the emergence of resistance in our population.

10.2 RECRUDESCENCE

Recrudescence of malaria was traditionally defined as occurrence of fever within 8 weeks of initial infection(55).5 patients had relapse of fever post 7 days of treatment with artesunate, and the mean duration of the relapse defervescence was 54 hours.

They did not fulfil the criteria for late clinical or parasitological failure(47),in view of absence of asexual ring forms in peripheral smears. However all of them had mature gametocyte stages, hence a low level parasitemia, not detectable by microscopy could be a possible explanation of recrudescence.

Rescue therapy was given in all the patients with higher dose of artesunate (3.2 mg/kg) with change in the partner drug to intravenous clindamycin, which led to rapid fever defervescence.

Ittarat et al (56)looked at a series of patients with recrudescence of *falciparum* infection and the association between host and parasite factors. They found that high parasite index at admission was found to have strong association with recrudescence rates. Presence of renal failure with uraemia at admission had a trend towards association.

All of the 5 patients were with severe malaria at admission, with high parasite burden and multi organ dysfunction, hence with recrudescence.

An extended follow up to 28 days or 42 days is required to further characterise the exact incidence of recrudescence and molecular PCR to detect low level parasitemia.

10.3 GAMETOCYTE CLEARANCE TIME AND TREATMENT FAILURE

Another interesting finding in the results is the difference in the median gametocyte clearance times between responders and patients with treatment failure. However the limitation to this parameter was all patients were followed up till day 7 and those patients who required hospitalisation were followed till discharge. It was found to be 3.5 days among responders and 7 days among non responders.

Studies have shown that gametocytemia in *falciparum* infections occur at 10 – 40 days after the parasitemia, and the longevity is documented to up to 21 days facilitating transmission(57).Piyaphanee et al studied the prevalence of gametocytemia post treatment with artemisinin combination therapy, and found 10% on day 7 and 4% at day 14(58) as compared to ours which was 28% at day 7 and 6% > day 7.

The prevalence of gametocytemia was found to be higher in our cohort when compared to the published studies assessing gametocyte clearance times. The prolonged gametocyte clearance might be the parasite factor enabling the spread of resistant strains.

10.4 DEMOGRAPHIC CHARACTERISTICS AND TREATMENT FAILURE

The patients in this cohort are young aged males (mean age: 37 years, range 17 – 75) and predominantly unskilled labourers mainly agriculture/field based workers. They are more predisposed to acquire malaria, in view of the close proximity to the vector breeding sites. The biting period of mosquitoes coincides with the peak activities of men predisposing them to acquire severe infection(59,60).However the distribution of patients with treatment failure was not different across the categories.

Age was found to have a statistically significant difference in patients who had adequate clinical and parasite clearance response compared with those patients with early treatment failure. Individuals with age more than 35 years have a strong trend towards treatment failure (OR 4.3, 95% CI 0.84 – 21).

Sowunmi et al(8) looked at factors associated with delayed parasite clearance in a cohort of African children, which showed age less than 2 was associated with delayed parasitological clearance. They attributed this to an impaired inbuilt immune response to enable efficient clearing of the parasites. The same hypothesis might be extrapolated in adults, as the production and migration of new B and T cells also show a decline with increasing age in adults, implying that the immune function declines with advancing age(61).

The gender and occupation increase the chances of exposure to the vector but is not a contributing factor for delayed clearance.

A trend for treatment failure was identified towards patients residing in Andhra Pradesh (OR– 1.210, 95% CI (0.34 – 4.24).The detailed geographic mapping of their location is mentioned in the results section. The trend could be explained by the following ways:

1) Andhra Pradesh has increasing rates of vector borne diseases commonly malaria, chikungunya, dengue and scrub typhus which occurs in areas surrounded by forests, which are now subjected to urbanisation and improper deforestation practices with dissemination of vectors(62).

2)The incidence of malaria is higher in Andhra when compared to Tamil Nadu.As of health status indicators in India – 2012,the state wise report of number of malaria cases, Andhra Pradesh reported 22723 as compared to Tamil Nadu, where the reported number was 15486(10).

3) Inadequate drug dosages and use of monotherapy.

4) Migrant population from the borders with acquired resistance to artemisinin.

Though we do not have evidence to substantiate the last two reasons, this trend indicates the need for urgent surveillance studies of parasite clearance and fever clearance times to be conducted in Andhra Pradesh especially Chittoor.

In Tamil Nadu, the patients with treatment failure are from Vellore, which has a common border with Chittoor, Andhra Pradesh, hence there is a likely chance of the spread of resistant parasites from the neighbouring state. This hypothesis requires further investigation, which would involve sequencing the genome of the parasite and clearance studies to be conducted in Andhra Pradesh.

10.5 CLINICAL PRESENTATION AND TREATMENT FAILURE

Malaria has a typical clinical presentation of high fever with chills and rigors associated with excessive malaise, headache and fatigue(63).Published series showed the presence of fever in more than 92% of the subjects followed by headache and malaise as common symptoms(64).In travellers returning from endemic countries, the presence of fever with sweating ,absence of abdominal pain and overall poor general health were found to be important clinical predictors of malaria(65).In our cohort, all patients reported fever with headache, which was the most common presenting symptom.

In a study of 1461 individuals by Dondorp et al(23), involving the South east Asian population, comparing the efficacy of artesunate over quinine in management of malaria, clinical jaundice was evident in 49% of the subjects. Clinical jaundice is the earliest sign of hepatic dysfunction, which can further progress to acute liver failure(66).In our study it was found to be 61.1%.

Pulmonary dysfunction is yet another complication of severe malaria. The incidence of the same published in case series by Sahu et al (67) comprising of hospitalised patients with *falciparum* malaria in intensive care was found to be 5.31%.In yet another series by Bruneel et al of 188 patients of severe *falciparum* malaria in intensive care(68) 14 patients had evidence of pulmonary oedema and 2 patients had acute lung injury.22.78 % of the patients in our study had symptoms of respiratory distress at presentation. The cause of respiratory distress is multi factorial, which could be due to fever, presence of metabolic acidosis or lung injury.10 patients had acute pulmonary oedema and 5 patients had acute respiratory distress syndrome in our study. The mechanism of lung injury which was earlier postulated is that of pulmonary micro vascular sequestration of parasitized red blood cells, however a hypothesis of post treatment immune mediated destruction of alveolar membrane is yet to be proven(69).

Cerebral malaria is defined as any alteration of mental status in a patient with malaria, in the absence of other potential causes to explain the same(64).The survivors usually do not have long term neurological sequelae.A significant proportion of 24.07% in our study had evidence of cerebral malaria at presentation. Though meningeal signs are reported to be rare(64),4 patients had terminal nuchal rigidity and 2 patients had generalised tonic clonic convulsions.1 patient had long term neurological sequelae in the form of persistent vegetative state. Brewster et al studied the predisposing factors for the development of long term sequelae in 604 Gambian children, and identified the severity of anaemia at presentation and seizures were found to be associated(70).

22.4% of the patients in the study reported systemic bleed, in the form of haematuria, haematemesis and epistaxis.2 patients had disseminated intravascular coagulation. The bleeding manifestations are attributed to the degree of thrombocytopenia. The bleeding manifestations are secondary to the endothelial injury caused by the host – parasite

interaction. Systemic inflammation precipitates a state of disseminated intravascular coagulation(64).

The clinical presentation was not found to vary among patients with adequate response and delayed clearance times. The severity of illness at presentation based on presence of fever, tachycardia, tachypnea, hypotension or hypoxia did not affect the parasite clearance or fever clearance.

On a study conducted in African children with uncomplicated malaria who received oral artesunate (71),presence of fever and vomiting was associated with increased risk of delayed clearance, secondary to decreased bio availability of the drug. Currently there is scarcity of evidence correlating these parameters with artesunate.

10.6 HAEMATOLOGICAL PARAMETERS AND TREATMENT FAILURE

Patil et al (72)in their retrospective study of patients with *falciparum* malaria from Central India reported 46.8% of anaemia and only 2.12% of thrombocytopenia. Gupta et al looked at prevalence of thrombocytopenia in patients with *vivax* and *falciparum* infections, and found it to be 77.7% in *falciparum* infection.

In our study we found 92.4 % of patients having thrombocytopenia and 51.8% have anemia.The proportion of patients who required product support was 27.7%,which was comparable to the published case series on severe malaria(67,68,73).

In the univariate analysis, though the severity of anaemia and thrombocytopenia was found to higher in the group with delayed parasitological clearance, the difference was not found to be statistically significant. However this was limited by the low sample size, which was probably under powered to study this difference.

In patients with delayed fever defervescence, thrombocytopenia was found to be significantly severe with a mean difference of 32684.3/cu mm (95% CI: 7.447 - 65361.24, p value; 0.053).

In the multivariate logistic regression analysis as well as in the boot strap analysis, presence thrombocytopenia or anaemia was not found to have an association with treatment failure.

It has been shown in animal models(murine),clearance of parasites(*Trypanasoma cruzi*) require a complement mediated immune activation and adequate platelets to clear the infection(74).Thrombocytopenia was found to significantly delay the parasite clearance.

In *falciparum* infection, the pathogenesis is a close interface between the immune and haematological system and platelets form the infrastructure on which the host directs the response. The relationship between delayed parasite clearance and these haematological alterations are yet to be deciphered.

10.7 PARASITE INDEX AT ADMISSION AND TREATMENT FAILURE

The mean parasite index of this cohort was 5.51 %(0.1% – 48%: SD – 11.4) indicating hyperparsitemic. Hyperparasitemic is a indirect marker to indicate host immunity(75,76). In low endemic settings, the host immunity to malarial parasites (innate as well as acquired) is poor. Hence inoculation of parasites, with poor host immune response leads to excessive multiplication in the host, leading to hyperparasitemia.

Stepniewska et al pooled the available data of parasite clearance times from multiple therapeutic efficacy studies conducted in different geographical territories to identify factors which determine the therapeutic responses(48). This was a large series involving 18,699 patients and it revealed ,less than 5% of patients with low level parasitemia at admission (<100,000 parasites/µL) had detectable parasitemia on day 3, compared to 25% of patients with hyperparasitemia (>100,000 parasites/µL)(p <0.001).

In a study from Thailand(56),which was a retrospective study of 104 patients with *falciparum* malaria, of which 32 patients had proven recrudescence.Hyperparasitemia at admission, as defined as parasite density >10,000 micro/litre, was found to have 9 times higher likelihood of recrudescence.

Univariate analysis comparing the parasite index at admission between the patients with adequate response and delayed clearance, showed higher parasite index in patients with delayed clearance (p value – 0.027).

Multi variate logistic regression analysis also yielded a positive likelihood ratio of 1.063 (95% CI: 0.98 – 1.14, p value – 0.125) .The difference was not found to be statistically significant, but there was trend towards association.

Boot strap analysis failed to demonstrate any significant association with delayed clearance. This might be confounded with the lesser numbers of patients with low level parasitemia, leading to an unequal numbers in both arms for accurate comparison.

Hyperparasitemia is an important factor for emergence of 'de novo' resistance mutations. The presence of hyperparasitemia when compounded with inadequate antimalarial exposure accelerates emergence and spread of resistance strains(77).

As hyperparasitemia is associated with delay in parasite clearance times, the currently adopted method is calculation of parasite clearance half life(78). This is calculated from the slope of the clearance curve got by plotting the log of parasitemia as measure by the parasite density against time. This will be an independent measure, not affected by the pre-treatment parasite density. The non availability of parasite density is a potential limitation in our study.

10.8 ACUTE KIDNEY INJURY AND TREATMENT FAILURE

Acute kidney injury(AKI) is a common finding in severe *falciparum* malaria(73,79–81). The incidence of AKI varies from <1% to 40 % in *falciparum* malaria(81).

The renal manifestations occur in two main forms:

1) Chronic malarial nephropathy – predominantly seen in the African population, in view of continuous exposure to the pathogen

2)Acute kidney injury, which predominates with *falciparum* infections in South East Asia(79).

The pathogenesis of acute kidney injury in malaria is multifactorial.

The commonly postulated mechanisms include obstruction of the microvasculature by parasitized red blood cells(sequestration),immune mediated glomerular and tubular damage,pre renal injury secondary to sepsis induced hypo perfusion and tissue hypoxia(64,79,81–84). In a large case series of AKI in malarial patients by Saravu et al,direct bilirubin was found to be a risk factor for renal dysfunction(82).

In our study, acute kidney injury was seen in 53.70 %(n = 29), with 55% having prerenal azotemia which was comparable to other studies from India. Haemodialysis requirement was 7% in our study, which was low when compared to the other published series(67,80,82,83).

Univariate analysis revealed significant difference in the serum creatinine and urea levels between patients who responded adequately and in those with treatment failure. The mean difference was found to be statistically significant. Multivariate logistic regression models and boot strap analysis revealed a 2 times higher risk of treatment failure in the presence of

acute kidney injury; it was not of statistical significance. Currently there is a scarcity of evidence on association between presence of multi organ dysfunction and treatment failure.

Acquired host immunity is currently identified to be the only factor deciding the clearance of parasites(85–87).This varies based on the endemicity of the region to malaria. Higher immunity accelerates clearance.

10.9 ACUTE HEPATIC INJURY AND TREATMENT FAILURE

The incidence of liver involvement in *falciparum* infections have been found to vary from 2.58% to 68%(66,88–90).The most common direct attributable causes include that of malarial hepatitis which is classically described as a 3 or more fold elevation of liver enzymes in the presence of *falciparum* infection, with an excellent response to antimalarial therapy and secondly by intravascular haemolysis of parasite laden red blood cells(88,91).

In our study 75.9% of the patients had hepatic dysfunction, most commonly unconjugated hyperbilirubinemia with or without transaminitis, which is higher when compared to all the published case series in India(66,67,72,73,88–90).

Univariate analysis revealed the presence of unconjugated hyperbilirubinemia and elevated SGPT >SGOT to be associated with treatment failure. The difference was found to be statistically significant. Hypoalbuminemia was strongly associated with delay in fever defervescence (p value – 0.006).This might be explained by the host immune response to the severity of infection, causing decrease in albumin, as it is a negative acute phase reactant.Multi variate regression analysis showed a trend towards association of direct bilirubin to treatment failure (OR -1.449; 95%CI 0.69 – 1; p – 0.318), however the difference was not statistically significant.

10.10 DRUG CONCENTRATIONS OF ARTESUNATE AND DIHYDROARTEMISININ AND TREATMENT FAILURE

Intravenous artesunate is the recommended first line agent in management of severe falciparum malaria(2).Evidence from African as well as South east Asian population holds artesunate higher up when compared to quinine in terms of its excellent efficacy as well as the rapidity of parasite clearance(22,23).

Our study subjects were administered intravenous artesunate at mean dose of 2.4 mg/kg and they achieved the plasma concentrations in a prompt manner. Very high plasma levels of artesunate and dihydroartemisinin were reached. The C_{max} (maximum concentration) for artesunate was reached at 5 minutes and for dihydroartemisinin at 12 minutes, indicating a rapid conversion of artesunate to its active metabolite. This was also evident from other published literature in the world, which shows the T_{max}(time to maximum concentration for artesunate to be less than 15 minutes and for dihydroartemisinin to be less than 25 minutes(92–96).

The drug cleared rapidly from the circulation, which was evident by the undetectable serum concentrations of artesunate as well its metabolite dihydroartemisinin by 120 minutes. In a review if pharmacokinetics of artesunate compounds by Morris et al(93),the clearance for artesunate was found to be 2 – 3l/kg/hour and for dihydroartemisinin was 0.5 – 1.5l/kg/hour. In comparison to this our study population had higher rates of clearance of 4.95 and 3.41 litre/kg/hour respectively.

The maximum concentration (C_{max}) of artesunate and dihydroartemisinin to a dose of 2.4mg/kg was low in our cohort when compared to the published studies on patients with *falciparum* malaria.

The pharmacokinetic values comparing our cohort to different ethnicity are tabulated in table 24.

Table 24 - COMPARISON OF INTRAVENOUS ARTESUNATE AND DHA PHARMACOKINETICS IN DIFFERENT ETHNICITIES

EVIDENCE(ETHNICITY)	AS C_{max}	DHA C_{max}	AS AUC	DHA AUC	Clearance l/kg/hr[AS]	Clearance l/kg/hr[DHA]
PRESENT STUDY(INDIAN)	5867	573.33	650.45	884.80	5.085	3.72
Byakika et al(97)(AFRICAN)	3260	3140	727	3492	180	32.25
Batty et al(98)(VIETNAM)	-	931	2980	8360	2.33	0.75
Li et al(99)(KENYA)	28558	2932	1878	3543	1.7	0.73
Newton et al(96)(THAILAND)	130	605	49	418	6.4	5.6

4 studies were identified from different ethnicity, which assessed the pharmacokinetics of artesunate and dihydroartemisinin.

One potential limitation in this comparison was that all these studies used intravenous artesunate as a bolus of 2.4 mg/kg, and the maximum concentration was obtained at the end of 2 minutes, when compared to our study where the maximum concentration was measured at the end of 5 minutes. In view of the rapid metabolism and half life of intravenous artesunate, the post 5 minute concentration, would be lower when compared to post 2 minute concentration. This would have falsely underestimated the maximum concentrations.

An interesting trend to note is the rapidity in clearance of the drug in our population. Our clearance values are much higher when compared to other studies, which shows a significant variation in the pharmacogenomics when compared to other ethnicities.

The question of whether our population requires higher dosing at baseline, needs to be answered through further research taking into account the proportion of treatment failure rates as well as the pharmacogenomics of the drug.

To ensure adequate availability of pure drug in the compounds given to the patients we went ahead and estimated the concentration of pure drug in the usual compound given to our patients and we found that it was within the prescribed normal limits.

Inter individual variation was noted between the concentrations of 4 hour AUC exposure and maximum concentrations (C_{max}). In a study done on 17 patients with severe *falciparum* malaria in Thailand, more than 10 fold inter patient variability was found in the drug concentrations, leading to the recommendation of minimum dosage of 2.4 mg/kg in treatment of severe malaria(96).Previously published studies elaborating the pharmacokinetics of artesunate in severe malaria, did not find any factors which altered drug concentrations or metabolism. (93,97,100).

In our study, we compared the parasite index at admission with the mean AUC $^{(0\text{-}240)}$ of artesunate and dihydroartemisinin. Both the concentrations were found to be lower in patients with hyperparasitemia (PI>2%).This difference was found to be statistically significant. Hence in view of this significant difference of drug concentrations in patients with severe malaria, a higher dosing regimen is to be considered in patients with higher parasite burden. White et al ,on their review on factors causing selection of 'de novo highly resistant mutant strains', have identified that low dosing regimens in hyperparasitemic individuals to contribute to the acquisition of resistance mutation and selection of the same to cause spread of resistance(77).

The metabolism of artesunate to dihydroartemisinin occurs in the liver, where the metabolites get glucuronidated and partly excreted in bile through faeces and partly cleared by urine(94,101).In our study, we compared the drug concentrations between subjects with acute kidney injury and normal renal function. The artesunate concentration was found to be lower in patients with AKI, but the concentrations of the metabolite dihydroartemisinin was higher in those subjects. This difference was not found to be statistically significant. This observation infers that there exists a difference in the concentration of the metabolite, hence the need for renal dose adjustment. There are potential dose related adverse effects described in literature which include delayed haemolysis and neurotoxicity(102–105). Hence the benefits of dose adjustment are to be interpreted based on long term follow up of the occurrence of side effects.

Multiple landmark trials as well as small scale studies comparing the drug concentrations to parasite clearance have not established any difference in the drug levels between those with treatment failure and responders(96,97,106).In our study we found lower levels of artesunate and its metabolite dihydroartemisinin in patients with treatment failure, with a trend towards statistical significance. However the unequal distribution in both arms of comparison is a potential limiting factor in our population, for a definite conclusion.

In the future, a larger study with equal numbers for comparison, with 2 minute duration of IV bolus, would be necessary for comparison between ethnicity and to clarify the pharmacogenomics and its clinical implications.

11. CONCLUSIONS

Artemisinin resistance is an emerging threat in the Indian Subcontinent. The median fever clearance time was 24 hours and median parasite clearance time was 36 hours, indicating the high efficacy and rapidity of action of artemisinin in management of severe malaria.

The incidence of treatment failure to ACT (artesunate+doxycyline/artemether+lumefantrine) in this cohort involving South Indian population with *falciparum* malaria was found to be 25.5%. The proportion of recrudescence was 9.3%, requiring rescue therapy with higher dose of intravenous artesunate and change in the partner drug to clindamycin.The median gametocyte clearance time was higher in patients with treatment failure, indicating a higher risk of transmission of resistant parasites to the community.

The drug concentrations of artesunate and metabolite dihydroartemisinin (DHA) for a mean dose of 2.4 mg/kg were lower in our cohort when compared to other cohorts from Vietnam, Cambodia and Thailand. The maximum serum concentrations are achieved in 5 minutes of dosing and is metabolised to DHA within 12 minutes of intravenous administration. The drug undergoes rapid clearance as evident by no detectable levels by 2 hours of dosing. Lower serum concentrations of the drugs were seen in hyperparsitemic individuals.

A trend was identified towards Andhra Pradesh for origin of treatment resistance, especially in the state borders with Tamil Nadu. Higher age, presence of acute kidney injury, hyperbilirubinemia, metabolic acidosis and hyperparasitemia were found to be associated with treatment failure in the univariate analysis. Multivariate analysis revealed only age to be a risk factor for treatment failure. The drug levels were similar between responders and treatment failure.

12. LIMITATIONS

1) The sample size was small, in view of the patient recruitment in the tertiary care referral centre. A community based study would have yielded more numbers. Other potential reason could be the fall in the number of malaria cases due to intense preventive and vector control measures in the state.

2) Calculation of parasite density and parasite clearance half lives is a better indicator of treatment failure than the day 3 parasitemia rates. The estimation of parasite density for our patients requires further validation.

3) The unequal number for comparison in the pharmacokinetic studies is a limitation to make any standard conclusion.

4)More frequent sampling would have accurately estimated the clearance times, as a twelfth hourly estimate has a higher chance to over estimate clearance times, when compared to sixth or eighth hourly assessments.

13. BIBLIOGRAPHY

1. Dondorp AM, Fairhurst RM, Slutsker L, Macarthur JR, Breman JG, Guerin PJ, et al. The threat of artemisinin-resistant malaria. N Engl J Med. 2011 Sep 22;365(12):1073–5.

2. WHO | World Malaria Report 2013 [Internet]. WHO. [cited 2014 Aug 3]. Available from: http://www.who.int/malaria/publications/world_malaria_report_2013/en/

3. Status_rep_artemisinin_resistance_jan2014.pdf [Internet]. [cited 2014 Aug 3]. Available from: http://www.wpro.who.int/world_health_day/2014/Status_rep_artemisinin_resistance_jan2014.pdf

4. WHO | Global report on antimalarial efficacy and drug resistance: 2000-2010 [Internet]. WHO. [cited 2014 Jul 24]. Available from: http://www.who.int/malaria/publications/atoz/9789241500470/en/

5. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, et al. Artemisinin Resistance in Plasmodium falciparum Malaria. N Engl J Med. 2009;361(5):455–67.

6. Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM, et al. Evidence of artemisinin-resistant malaria in western Cambodia. N Engl J Med. 2008 Dec 11;359(24):2619–20.

7. Spread of Artemisinin Resistance in Plasmodium falciparum Malaria. N Engl J Med. 2014 Jul 30;

8. 1475-2875-13-284.pdf [Internet]. [cited 2014 Aug 3]. Available from: http://www.malariajournal.com/content/pdf/1475-2875-13-284.pdf

9. NVBDCP | National Vector Borne Disease Control Programme [Internet]. [cited 2014 Sep 14]. Available from: http://nvbdcp.gov.in/malaria3.html

10. Health Status Indicators-2012.pdf [Internet]. [cited 2014 Sep 14]. Available from: http://cbhidghs.nic.in/writereaddata/mainlinkFile/Health%20Status%20Indicators-2012.pdf

11. Disease Burden - Malaria [Internet]. [cited 2014 Sep 14]. Available from: http://www.tnhealth.org/dph/dphdbmal.php

12. Dhingra N, Jha P, Sharma VP, Cohen AA, Jotkar RM, Rodriguez PS, et al. Adult and child malaria mortality in India: a nationally representative mortality survey. The Lancet. 2010 Nov;376(9754):1768–74.

13. Volkman SK, Sabeti PC, DeCaprio D, Neafsey DE, Schaffner SF, Milner DA, et al. A genome-wide map of diversity in Plasmodium falciparum. Nat Genet. 2007 Jan;39(1):113–9.

14. Oh SS, Chishti AH, Palek J, Liu SC. Erythrocyte membrane alterations in Plasmodium falciparum malaria sequestration. Curr Opin Hematol. 1997 Mar;4(2):148–54.

15. Rogerson SJ, Tembenu R, Dobaño C, Plitt S, Taylor TE, Molyneux ME. Cytoadherence characteristics of Plasmodium falciparum-infected erythrocytes from Malawian children with severe and uncomplicated malaria. Am J Trop Med Hyg. 1999 Sep;61(3):467–72.

16. WASSMER SC, TAYLOR T, MacLENNAN CA, KANJALA M, MUKAKA M, MOLYNEUX ME, et al. Platelet-induced clumping of Plasmodium falciparum-infected erythrocytes from

Malawian patients with cerebral malaria - possible modulation in vivo by thrombocytopenia. J Infect Dis. 2008 Jan 1;197(1):72–8.

17. Francischetti IMB, Seydel KB, Monteiro RQ. Blood Coagulation, Inflammation, and Malaria. Microcirculation. 2008 Feb 1;15(2):81–107.

18. Moody A. Rapid diagnostic tests for malaria parasites. Clin Microbiol Rev. 2002 Jan;15(1):66–78.

19. Delves M, Plouffe D, Scheurer C, Meister S, Wittlin S, Winzeler EA, et al. The Activities of Current Antimalarial Drugs on the Life Cycle Stages of Plasmodium: A Comparative Study with Human and Rodent Parasites. PLoS Med. 2012 Feb 21;9(2):e1001169.

20. 9789241547925_eng.pdf [Internet]. [cited 2014 Sep 1]. Available from: http://whqlibdoc.who.int/publications/2010/9789241547925_eng.pdf

21. Adjuik M, Babiker A, Garner P, Olliaro P, Taylor W, White N, et al. Artesunate combinations for treatment of malaria: meta-analysis. Lancet. 2004 Jan 3;363(9402):9–17.

22. Dondorp AM, Fanello CI, Hendriksen ICE, Gomes E, Seni A, Chhaganlal KD, et al. Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. Lancet. 2010 Nov 13;376(9753):1647–57.

23. Dondorp A, Nosten F, Stepniewska K, Day N, White N, South East Asian Quinine Artesunate Malaria Trial (SEAQUAMAT) group. Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. Lancet. 2005 Sep 27;366(9487):717–25.

24. Sinclair D, Donegan S, Isba R, Lalloo DG. Artesunate versus quinine for treating severe malaria. Cochrane Database Syst Rev. 2012;6:CD005967.

25. Meshnick SR, Taylor TE, Kamchonwongpaisan S. Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. Microbiol Rev. 1996 Jun;60(2):301–15.

26. Meshnick SR, Taylor TE, Kamchonwongpaisan S. Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. Microbiol Rev. 1996 Jun;60(2):301–15.

27. Postma NS, Mommers EC, Eling WM, Zuidema J. Oxidative stress in malaria; implications for prevention and therapy. Pharm World Sci PWS. 1996 Aug;18(4):121–9.

28. Wu Y. How might qinghaosu (artemisinin) and related compounds kill the intraerythrocytic malaria parasite? A chemist's view. Acc Chem Res. 2002 May;35(5):255–9.

29. Meshnick SR. Artemisinin and Heme. Antimicrob Agents Chemother. 2003 Aug;47(8):2712–3.

30. Eckstein-Ludwig U, Webb RJ, Van Goethem IDA, East JM, Lee AG, Kimura M, et al. Artemisinins target the SERCA of Plasmodium falciparum. Nature. 2003 Aug 21;424(6951):957–61.

31. Morris CA, Duparc S, Borghini-Fuhrer I, Jung D, Shin C-S, Fleckenstein L. Review of the clinical pharmacokinetics of artesunate and its active metabolite dihydroartemisinin following intravenous, intramuscular, oral or rectal administration. Malar J. 2011 Sep 13;10:263.

32. Batty KT, Le AT, Ilett KF, Nguyen PT, Powell SM, Nguyen CH, et al. A pharmacokinetic and pharmacodynamic study of artesunate for vivax malaria. Am J Trop Med Hyg. 1998 Nov;59(5):823–7.

33. Batty KT, Thu LT, Davis TM, Ilett KF, Mai TX, Hung NC, et al. A pharmacokinetic and pharmacodynamic study of intravenous vs oral artesunate in uncomplicated falciparum malaria. Br J Clin Pharmacol. 1998 Feb;45(2):123–9.

34. Ilett KF, Batty KT, Powell SM, Binh TQ, Thu LTA, Phuong HL, et al. The pharmacokinetic properties of intramuscular artesunate and rectal dihydroartemisinin in uncomplicated falciparum malaria. Br J Clin Pharmacol. 2002 Jan;53(1):23–30.

35. Newton PN, Barnes KI, Smith PJ, Evans AC, Chierakul W, Ruangveerayuth R, et al. The pharmacokinetics of intravenous artesunate in adults with severe falciparum malaria. Eur J Clin Pharmacol. 2006 Dec;62(12):1003–9.

36. Li Q, Cantilena LR, Leary KJ, Saviolakis GA, Miller RS, Melendez V, et al. Pharmacokinetic profiles of artesunate after single intravenous doses at 0.5, 1, 2, 4, and 8 mg/kg in healthy volunteers: a phase I study. Am J Trop Med Hyg. 2009 Oct;81(4):615–21.

37. Binh TQ, Ilett KF, Batty KT, Davis TM, Hung NC, Powell SM, et al. Oral bioavailability of dihydroartemisinin in Vietnamese volunteers and in patients with falciparum malaria. Br J Clin Pharmacol. 2001 Jun;51(6):541–6.

38. Ilett KF, Ethell BT, Maggs JL, Davis TME, Batty KT, Burchell B, et al. Glucuronidation of dihydroartemisinin in vivo and by human liver microsomes and expressed UDP-glucuronosyltransferases. Drug Metab Dispos Biol Fate Chem. 2002 Sep;30(9):1005–12.

39. Davis TM, Phuong HL, Ilett KF, Hung NC, Batty KT, Phuong VD, et al. Pharmacokinetics and pharmacodynamics of intravenous artesunate in severe falciparum malaria. Antimicrob Agents Chemother. 2001 Jan;45(1):181–6.

40. Stepniewska K, Taylor W, Sirima SB, Ouedraogo EB, Ouedraogo A, Gansané A, et al. Population pharmacokinetics of artesunate and amodiaquine in African children. Malar J. 2009 Aug 20;8(1):200.

41. Newton PN, Vugt M van, Teja-Isavadharm P, Siriyanonda D, Rasameesoroj M, Teerapong P, et al. Comparison of Oral Artesunate and Dihydroartemisinin Antimalarial Bioavailabilities in Acute Falciparum Malaria. Antimicrob Agents Chemother. 2002 Apr 1;46(4):1125–7.

42. Gueye CS, Newby G, Hwang J, Phillips AA, Whittaker M, MacArthur JR, et al. The challenge of artemisinin resistance can only be met by eliminating Plasmodium falciparum malaria across the Greater Mekong subregion. Malar J. 2014 Jul 27;13(1):286.

43. arupdate042012.pdf [Internet]. [cited 2014 Jul 23]. Available from: http://www.who.int/malaria/publications/atoz/arupdate042012.pdf

44. status-rep-artemisinin-resistance-sep2014.pdf [Internet]. [cited 2014 Sep 8]. Available from: http://www.who.int/malaria/publications/atoz/status-rep-artemisinin-resistance-sep2014.pdf?ua=1

45. Newton PN, Fernández FM, Plançon A, Mildenhall DC, Green MD, Ziyong L, et al. A Collaborative Epidemiological Investigation into the Criminal Fake Artesunate Trade in South East Asia. PLoS Med. 2008 Feb 12;5(2):e32.

46. Newton PN, McGready R, Fernandez F, Green MD, Sunjio M, Bruneton C, et al. Manslaughter by Fake Artesunate in Asia—Will Africa Be Next? PLoS Med. 2006 Jun 13;3(6):e197.

47. WHO | Methods for surveillance of antimalarial drug efficacy [Internet]. WHO. [cited 2014 Jul 24]. Available from: http://www.who.int/malaria/publications/atoz/9789241597531/en/

48. Stepniewska K, Ashley E, Lee SJ, Anstey N, Barnes KI, Binh TQ, et al. In vivo parasitological measures of artemisinin susceptibility. J Infect Dis. 2010 Feb 15;201(4):570–9.

49. WHO | Emergency response to artemisinin resistance in the Greater Mekong subregion. Regional framework for action 2013-2015 [Internet]. WHO. [cited 2014 Sep 8]. Available from: http://www.who.int/malaria/publications/atoz/9789241505321/en/

50. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, et al. Spread of artemisinin resistance in Plasmodium falciparum malaria. N Engl J Med. 2014 Jul 31;371(5):411–23.

51. Greenwood B. Treatment of Malaria — A Continuing Challenge. N Engl J Med. 2014 Jul 30;371(5):474–5.

52. Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois A-C, Khim N, et al. A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. Nature. 2014 Jan 2;505(7481):50–5.

53. Conrad MD, Bigira V, Kapisi J, Muhindo M, Kamya MR, Havlir DV, et al. Polymorphisms in K13 and Falcipain-2 Associated with Artemisinin Resistance Are Not Prevalent in Plasmodium falciparum Isolated from Ugandan Children. PLoS ONE [Internet]. 2014 Aug 21 [cited 2014 Sep 8];9(8). Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4140830/

54. Dondorp AM, Ringwald P. Artemisinin resistance is a clear and present danger. Trends Parasitol. 2013 Aug;29(8):359–60.

55. P A. Recurrent malaria - An enigma? Indian J Med Sci. 2000 Aug 1;54(8):325.

56. Ittarat W, Pickard AL, Rattanasinganchan P, Wilairatana P, Looareesuwan S, Emery K, et al. Recrudescence in artesunate-treated patients with falciparum malaria is dependent on parasite burden not on parasite factors. Am J Trop Med Hyg. 2003 Feb;68(2):147–52.

57. Smalley ME, Sinden RE. Plasmodium falciparum gametocytes: their longevity and infectivity. Parasitology. 1977 Feb;74(1):1–8.

58. Piyaphanee W, Krudsood S, Tangpukdee N, Thanachartwet W, Silachamroon U, Phophak N, et al. Emergence and Clearance of Gametocytes in Uncomplicated Plasmodium Falciparum Malaria. Am J Trop Med Hyg. 2006 Mar 1;74(3):432–5.

59. Parks W, Bryan J, others. Gender, mosquitos and malaria: implications for community development programs in Laputta, Myanmar. 2001;

60. gender_health_malaria.pdf [Internet]. [cited 2014 Sep 17]. Available from: http://www.who.int/gender/documents/gender_health_malaria.pdf

61. Montecino-Rodriguez E, Berent-Maoz B, Dorshkind K. Causes, consequences, and reversal of immune system aging. J Clin Invest. 2013 Mar 1;123(3):958–65.

62. N. Srinivasulu, et al.pdf [Internet]. [cited 2014 Sep 14]. Available from: http://www.ijcmas.com/Archives/vol-2-5/N.%20Srinivasulu,%20et%20al.pdf

63. Sadanand S. Malaria: An Evaluation of the Current State of Research on Pathogenesis and Antimalarial Drugs. Yale J Biol Med. 2010 Dec;83(4):185–91.

64. Trampuz A, Jereb M, Muzlovic I, Prabhu RM. Clinical review: Severe malaria. Crit Care. 2003;7(4):315–23.

65. D'Acremont V, Landry P, Mueller I, Pécoud A, Genton B. Clinical and laboratory predictors of imported malaria in an outpatient setting: an aid to medical decision making in returning travelers with fever. Am J Trop Med Hyg. 2002 May;66(5):481–6.

66. Baheti R, Laddha P, Gehlot RS. Liver involvement in falciparum malária-A histo-pathological analysis. J Indian Acad Clin Med. 2003;4(1):34–8.

67. Sahu S, Mohanty NK, Rath J, Patnaik SB. Spectrum of malaria complications in an intensive care unit. Singapore Med J. 2010 Mar;51(3):226–9.

68. Bruneel F, Hocqueloux L, Alberti C, Wolff M, Chevret S, Bédos J-P, et al. The Clinical Spectrum of Severe Imported Falciparum Malaria in the Intensive Care Unit. Am J Respir Crit Care Med. 2003 Mar 1;167(5):684–9.

69. Maguire GP, Handojo T, Pain MCF, Kenangalem E, Price RN, Tjitra E, et al. Lung Injury in Uncomplicated and Severe Falciparum Malaria: A Longitudinal Study in Papua, Indonesia. J Infect Dis. 2005 Dec 1;192(11):1966–74.

70. Brewster DR, Kwiatkowski D, White NJ. Neurological sequelae of cerebral malaria in children. Lancet. 1990 Oct 27;336(8722):1039–43.

71. Sowunmi A, Adewoye EO, Gbotsho GO, Happi CT, Sijuade A, Folarin OA, et al. Factors contributing to delay in parasite clearance in uncomplicated falciparum malaria in children. Malar J. 2010;9:53.

72. Patil V. Complicated falciparum Malaria in western Maharashtra. Trop Parasitol. 2012;2(1):49.

73. Krishnan A, Karnad DR. Severe falciparum malaria: an important cause of multiple organ failure in Indian intensive care unit patients. Crit Care Med. 2003 Sep;31(9):2278–84.

74. Umekita LF, Piazza RM, Mota I. Role of platelets and complement in the clearance of epimastigote forms of Trypanosoma cruzi. Braz J Med Biol Res Rev Bras Pesqui Médicas E Biológicas Soc Bras Biofísica Al. 1994 Oct;27(10):2391–9.

75. Doolan DL, Dobaño C, Baird JK. Acquired Immunity to Malaria. Clin Microbiol Rev. 2009 Jan 1;22(1):13–36.

76. Maire N, Smith T, Ross A, Owusu-Agyei S, Dietz K, Molineaux L. A Model for Natural Immunity to Asexual Blood Stages of Plasmodium Falciparum Malaria in Endemic Areas. Am J Trop Med Hyg. 2006 Aug 1;75(2 suppl):19–31.

77. White NJ, Pongtavornpinyo W, Maude RJ, Saralamba S, Aguas R, Stepniewska K, et al. Hyperparasitaemia and low dosing are an important source of anti-malarial drug resistance. Malar J. 2009;8:253.

78. Flegg JA, Guerin PJ, White NJ, Stepniewska K. Standardizing the measurement of parasite clearance in falciparum malaria: the parasite clearance estimator. Malar J. 2011 Nov 10;10(1):339.

79. Barsoum RS. Malarial Acute Renal Failure. J Am Soc Nephrol. 2000 Nov 1;11(11):2147–54.

80. Khan R, Quaiser S, Haque S. Malarial acute kidney injury: Prognostic markers. Ann Trop Med Public Health. 2013;6(3):280.

81. Mishra SK, Das BS. Malaria and acute kidney injury. Semin Nephrol. 2008 Jul;28(4):395–408.

82. Saravu K, Rishikesh K, Parikh CR. Risk Factors and Outcomes Stratified by Severity of Acute Kidney Injury in Malaria. PLoS ONE. 2014 Mar 13;9(3):e90419.

83. Shukla VS, Singh RG, Rathore SS, Usha. Outcome of malaria-associated acute kidney injury: a prospective study from a single center. Ren Fail. 2013 Jun 3;35(6):801–5.

84. Sitprija V. Nephropathy in falciparum malaria. Kidney Int. 1988 Dec;34(6):867–77.

85. Adam I, Elmardi KA, Malik EM. Predictors of antimalarial treatment failure in an area of unstable malaria transmission in eastern Sudan. Trans R Soc Trop Med Hyg. 2009 Jan 1;103(1):21–4.

86. Djimdé AA, Doumbo OK, Traore O, Guindo AB, Kayentao K, Diourte Y, et al. Clearance of Drug-Resistant Parasites as a Model for Protective Immunity in Plasmodium Falciparum Malaria. Am J Trop Med Hyg. 2003 Nov 1;69(5):558–63.

87. Dorsey G, Gasasira AF, Machekano R, Kamya MR, Staedke SG, Hubbard A. The Impact of Age, Temperature, and Parasite Density on Treatment Outcomes from Antimalarial Clinical Trials in Kampala, Uganda. Am J Trop Med Hyg. 2004 Nov 1;71(5):531–6.

88. Anand AC, Puri P. Jaundice in malaria. J Gastroenterol Hepatol. 2005 Sep;20(9):1322–32.

89. Murthy GL, Sahay RK, Sreenivas DV, Sundaram C, Shantaram V. Hepatitis in falciparum malaria. Trop Gastroenterol Off J Dig Dis Found. 1998 Dec;19(4):152–4.

90. Harris VK, Richard VS, Mathai E, Sitaram U, Kumar KV, Cherian AM, et al. A study on clinical profile of falciparum malaria in a tertiary care hospital in south India. Indian J Malariol. 2001 Jun;38(1-2):19–24.

91. Bhalla A, Suri V, Singh V. Malarial hepatopathy. J Postgrad Med. 2006 Dec;52(4):315–20.

92. Hanpithakpong W, Kamanikom B, Dondorp AM, Singhasivanon P, White NJ, Day NPJ, et al. A liquid chromatographic-tandem mass spectrometric method for determination of artesunate and its metabolite dihydroartemisinin in human plasma. J Chromatogr B. 2008 Dec 1;876(1):61–8.

93. Morris CA, Duparc S, Borghini-Fuhrer I, Jung D, Shin C-S, Fleckenstein L. Review of the clinical pharmacokinetics of artesunate and its active metabolite dihydroartemisinin following intravenous, intramuscular, oral or rectal administration. Malar J. 2011;10:263.

94. Navaratnam V, Mansor SM, Sit NW, Grace J, Li Q, Olliaro P. Pharmacokinetics of artemisinin-type compounds. Clin Pharmacokinet. 2000 Oct;39(4):255–70.

95. Newton P, Suputtamongkol Y, Teja-Isavadharm P, Pukrittayakamee S, Navaratnam V, Bates I, et al. Antimalarial bioavailability and disposition of artesunate in acute falciparum malaria. Antimicrob Agents Chemother. 2000 Apr;44(4):972–7.

96. Newton PN, Barnes KI, Smith PJ, Evans AC, Chierakul W, Ruangveerayuth R, et al. The pharmacokinetics of intravenous artesunate in adults with severe falciparum malaria. Eur J Clin Pharmacol. 2006 Dec;62(12):1003–9.

97. Byakika-Kibwika P, Lamorde M, Mayito J, Nabukeera L, Mayanja-Kizza H, Katabira E, et al. Pharmacokinetics and pharmacodynamics of intravenous artesunate during severe malaria treatment in Ugandan adults. Malar J. 2012 Apr 27;11(1):132.

98. Batty KT, Thu LT, Davis TM, Ilett KF, Mai TX, Hung NC, et al. A pharmacokinetic and pharmacodynamic study of intravenous vs oral artesunate in uncomplicated falciparum malaria. Br J Clin Pharmacol. 1998 Feb;45(2):123–9.

99. Li Q, Remich S, Miller SR, Ogutu B, Otieno W, Melendez V, et al. Pharmacokinetic evaluation of intravenous artesunate in adults with uncomplicated falciparum malaria in Kenya: a phase II study. Malar J. 2014 Dec 1;13(1):1–9.

100. Davis TM, Phuong HL, Ilett KF, Hung NC, Batty KT, Phuong VD, et al. Pharmacokinetics and pharmacodynamics of intravenous artesunate in severe falciparum malaria. Antimicrob Agents Chemother. 2001 Jan;45(1):181–6.

101. Artemisinin and Derivatives Pathway, Pharmacokinetics [Internet]. PharmGKB. [cited 2014 Sep 19]. Available from: http://www.pharmgkb.org/pathway/PA165378192

102. Rolling T, Agbenyega T, Issifou S, Adegnika AA, Sylverken J, Spahlinger D, et al. Delayed hemolysis after treatment with parenteral artesunate in African children with severe malaria--a double-center prospective study. J Infect Dis. 2014 Jun 15;209(12):1921–8.

103. Paczkowski MM, Landman KL, Arguin PM, Epidemic Intelligence Service, CDC. Update on cases of delayed hemolysis after parenteral artesunate therapy for malaria - United States, 2008 and 2013. MMWR Morb Mortal Wkly Rep. 2014 Aug 29;63(34):753–5.

104. Nontprasert A, Pukrittayakamee S, Nosten-Bertrand M, Vanijanonta S, White NJ. Studies of the neurotoxicity of oral artemisinin derivatives in mice. Am J Trop Med Hyg. 2000 Mar;62(3):409–12.

105. Efferth T, Kaina B. Toxicity of the antimalarial artemisinin and its dervatives. Crit Rev Toxicol. 2010 May;40(5):405–21.

106. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, et al. Artemisinin resistance in Plasmodium falciparum malaria. N Engl J Med. 2009 Jul 30;361(5):455–67.

ANNEXURES

1) PATIENT INFORMATION SHEET

2) PATIENT CONSENT FORMS

3) DATA ABSTRACTION SHEET

4) DATA WORKSHEET

ANNEXURE 1

PATIENT INFORMATION SHEET

TITLE: ASSESSMENT OF TREATMENT FAILURE WITH ARTEMISININ COMBINATION THERAPY IN PLASMODIUM FALCIPARUM MALARIA

1) ABOUT THE STUDY:

This study is done to assess whether the drug called artemisinin,which is a antimalarial drug is effective in clearing the malarial infection from the body as rapidly as it used to clear when it was introduced,that is within 48 hours.This study will also look at the number of patients who have delayed clearance of parasites from the blood following administration of this drug.

2) PARTS OF THE STUDY:

When you are identified to be an eligible candidate to be a part of the study, you will be approached by the doctor conducting the study. You will be asked a few questions about the details of your present illness and about your other underlying problems. You will be subjected to a thorough physical examination by the doctor. Following which you will be initiated on the medicines.Blood samples to check for parasites will be done twice daily till you clear the infection. On day 3 and day 7 you will be advised to follow up with a parasite count and index.

3) RISKS INVOLVED IN PARTICIPATION:

There are no risks involved in your participation in this study. You will receive the same treatment as given to a similar patient who is diagnosed with malaria and not a part of the study.

4)BENEFITS INVOLVED IN PARTICIPATION:

Decreasing effectiveness of this antimalarial medicine artesunate is a potential threat to emergence of resistance.The information we collect in this study will help us to identify the percentage of patients having early failure to therapy,hence can help in making guidelines to halt the process causing resistance and as well as to contain it.

5)RESULTS OF THE STUDY:

The results of the study will be compiled and will be put forth to the general information of public and professionals in the form of publications,posters or as presentations in conference.The information regarding you will only be handled by the doctor who conducts the study and others connected with the study.

If you have more queries you can contact:

Dr.T.Angel Miraclin

PG registrar, General Medicine

CMC,Vellore

ANNEXURE 1(b)

PATIENT INFORMATION SHEET FOR PARTICIPANTS OF THE STUDY OF PHARMACOKINETICS OF ARTEMISININ(AS/DHA)

PROJECT TITLE : ASSESSMENT OF TREATMENT FAILURE TO ARTEMISININ COMBINATION THERAPY IN PLASMODIUM FALCIPARUM MALARIA

1)What is this study about?

Following the diagnosis of severe malaria and hospitalisation,you will be initiated on Intravenous artesunate with oral doxycycline.There is a study which will be done to assess the declining effectiveness of this drug to clear parasites within 48 hours. On day 3 you will be enrolled in the second part of the study which will monitor the levels of this drug in your body.In exception to the routine we would like to assess the levels of artesunate and dihydroartemisinin in your blood three days after you are started on the medicine.Measurement of the drug levels,will reveal the actual difference in the levels between you and the other patients.Also your levels taken at different time periods will help to understand the variability of levels of artesunate and dihydroartemisinin in your blood at different selected time points.

2)How is it done?

Following hospitalisation you will have a thorough physical examination and blood tests will be sent as a part of the standard of care by your treating physician.You will be started on IV artesunate which you will receive 3 doses at 2.4mg/kg each at 0,12 and 24 hours on day 1,following that you will receive a once daily dosage. On day 3 you will be shifted to the clinical pharmacology laboratory by the doctor conducting this study. We will take a sample of blood(2 ml) for testing the levels of artesunate and dihydroartemisinin before giving the

day 3 dose.Following which an insyte will be inserted in the opposite arm to that of the insyte which delivers the drug.Artesunate will be given at dose of 2.4 mg/kg of your body weight.Then 2 ml of blood will be collected from you at regular intervals to get a total of 10 samples.Using the 10 samples ,we will have an understanding of the amount of artesunate and dihydroartemisinin present in your blood in between the two dosing periods.

3)Expenses involved?

You will not be required to pay for any of the level tests for artesunate or dihydroartemisinin at any point of time.

4)Risks Involved?

There are no potential risks involved in participating in this study.

Participation in this study is entirely voluntary and you may say no to taking part at any point of time.If you decide you do not want to be included in the study this will not affect your further management in this hospital.

In case of more queries:

Dr.T.Angel Miraclin

PG Registrar,General Medicine

CMC,Vellore.

ANNEXURE 2

Study Title: Assessment of treatment failure to artemisinin combination therapy in treatment of Severe malaria.

Subject's Initials: _____ Subject's Name: _____

Date of Birth / Age:_____

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

(iii) I understand that the Sponsor of the clinical trial, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]

(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s) [ ]

(v) I agree to take part in the above study. [ ]

Signature (or Thumb impression) of the Subject/Legally Acceptable Representative:_____

Date: _____/_____/_____

Signatory's Name: _____

Signature of the Investigator: _____

Date: _____/_____/_____

Study Investigator's Name: _____

ANNEXURE 3

PROFORMA – ASSESSMENT OF TREATMENT FAILURE TO ARTEMISININ COMBINATION THERAPY PRESENTING TO A TERTIARY CARE HOSPITAL:

----------Name: Age: Sex:M/F Date of visit:

Occupation: HW/STU/BUS/AGRI/LAB/OFFICE/..... H.NO:

State:TN/AP/OTHERS Ph NO: IP/OP Ward: I/C/E/MTS4 /MHDU/MICU/ED

RECENT TRAVEL:Y/N MONO/MIXED

HTN	Yes/No	CKD	Yes/No	CVA	Yes/No	CLD	Yes/No
DM	Yes/No	IHD	Yes/No	HIV	Yes/No	COPD	Yes/No

SYMPTOM	DURATION		D-0	D-3	D- 7
FEVER			Yes/No	Yes/No	Yes/No
ALTEREDSENSORIUM			Yes/No	Yes/No	Yes/No
RESPI DISTRESS			Yes/No	Yes/No	Yes/No
CONVULSIONS	Yes/No	GTCS/FOCAL	Yes/No	Yes/No	Yes/No
BLEEDING	Yes/No	EPI/ORAL/UGI/LGI/ UT	Yes/No	Yes/No	Yes/No
HEADACHE			Yes/No	Yes/No	Yes/No
JAUNDICE			Yes/No	Yes/No	Yes/No

PR	/min	SPO 2	%	ICTERUS	Yes/No	LIVER	Yes/No
BP	/ mm HG	TEM		CREPITATIONS	Yes/No	SPLEEN	Yes/No
RR	/min	GCS	/15	DROWSINESS	Yes/No	NECKSTIFF	Yes/No

Hb		Glucose		TB/DB	/
TC		Creat/Urea	/	Prot/Alb	/
Neutrophil		Na/K/HCO3	/ /	OT/PT	/
Lymphocytes		pH		A.PHOS	
Left shift	Yes/No BF - %	Bacteremia	Yes/No	CPK	
Platelets		Organism		CKMB/TRP	
Procal		CSF TC		N/L/GLU/PROT	/ / /

CXR	Normal/Consolidation/Pleural effusion/Alveolar infiltrates/post infectious sequelae
USG	Hepatomegaly/Splenomegaly/Free fluid/GB wall thickening/

CT BRAIN	Meningeal enhancement/Infarct/bleed

Supports	Oxygen	NIV	IV	Dialysis	Inotropes 1/2/3			
Duration					Dopa	Norad	Adr	vaso
TRANSFUSION	PC=	PRC=	FFP=	CRYO=				
UGI SCOPY	Yes/No	Colonoscopy	Yes/No	Findings:	Nosocomial infec tion– Yes/No VAP/UTI/CRBSI/Thrombophlebitis			

DYSFUN	**LEVEL 0**	**LEVEL I**	**LEVEL II**	**LEVEL III**
GCS	14 – 15(0)	10 – 13(1)	7 – 9(3)	0 – 6(5)
Urea Creatinine UrineOutput	10-36(0) 0.6 – 1.2(0) 0.75 -3.9(0)	37-59(1) 1.3 – 1.9(1) 0.5-0.75(1)	60-119(3) 2 -4.9(3) 0.4 – 0.5(3)	>120(5) >5(5) <0.5(5)
HR SYST BP	51 – 119(0) 90 – 160(0)	120-139(1) 70 – 89(1)	>140 OR <51(3) 41 – 59(3)	
RR	20 -30(0)	31 – 40(1)	>41(3)	
Hb(gm/dl) TLC(/cu mm) Platelet	10 -13.9(0) 4001-16000(0) 80K – 2,50K(0)	7 -9.9(1) 2001-4000,>10000(1) <80000(1)	<7(3) <2000(3)	
S.BILIRUBIN	<2(0)	>2(1)		
GLUCOSE(mg/dl)	60 -110(0)	<60(1)		

SCORE	**RISK**
<5	LOW
6 – 11	INTERMEDIATE
>12	HIGH

REGIMEN: [Artesunate+doxycycline] [Artemether+lumefantrine] [Artesunate+clindamycin]
EMPIRICAL ANTIBIOTIC: YES/NO **If yes,what antibiotic**:

If treatment failure- regimen

FEVER DOCUMENTATION

DAY	12AM -6AM	6AM – 12PM	12PM - 6PM	6PM -12AM	**<24hr**	Yes/no
1	Yes/No	Yes/No	Yes/No	Yes/No	**24-48h**	Yes/no
2	Yes/No	Yes/No	Yes/No	Yes/No	**48-72h**	Yes/no
3	Yes/No	Yes/No	Yes/No	Yes/No	**>72h**	Yes/no
7	Yes/No	Yes/No	Yes/No	Yes/No		

PARASITES DOCUMENTATION

DAY	RINGSMORNING	PI	EVENING	PI	GAMETOCYTES	**<24hr**	Yes/no
1	Yes/No		Yes/No		Yes/No	**24-48h**	Yes/no
2	Yes/No		Yes/No		Yes/No	**48-72h**	Yes/no

3	Yes/No		Yes/No		Yes/No	**>72h**	Yes/no
7	Yes/No		X		Yes/No		

DURATION OF ADMISSION:

OUTCOMES:ETF/LTF/ACPR/DEATH

IN CASE OF LTF

DAY	SEVERE MALARIA	Fever	Parasites(rings)	Gametocytes
14	Yes/no	Yes/no	Yes/no	Yes/no
21	Yes/no	Yes/no	Yes/no	Yes/no
28	Yes/no	Yes/no	Yes/no	Yes/no

INCLUDED FOR PHARMACOKINETIC STUDIES: YES/NO

BLOOD SAMPLES STORED FOR GENETIC ANALYSIS: YES/NO